Krijgslaan 281-S8
9000 Gent

Faculteit Wetenschappen
Academiejaar 2002-2003

Protozoan communities in intertidal estuarine sediments, their dynamics and trophic interactions

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Ilse Hamels

Proefschrift ingediend tot het behalen van de
graad van Doctor in de Wetenschappen (Biologie)

Promotor: Prof. Dr. Wim Vyverman

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Wie niets weet, twijfelt nergens aan.

De Druivelaar

Savoir bien, c'est toute l'affaire, car savoir tout est impossible

Alain, Frans filosoof

De studie van de wiskunde is als zeep voor kledingstukken.
Zij wast het vuil weg en verwijdert de vlekken

Ibn Khaldoen, 14de-eeuwse Noord-Afrikaanse historicus

A closer look at the rules of the game in the fast lane of the microbial realm would require an in situ computerized telemicroscope. Could such an instrument do for microbial ecology what Galileo's telescope did for astronomy? After all, there are orders of magnitude more bacteria in the ocean than there are stars in the Universe

Victor Smetacek, Nature vol. 419, 2002

Contents

Dankwoord

Voordat ik me aan een vreugdedansje waag zou ik nog graag al de mensen in de bloemetjes zetten die op de ene of de andere manier hebben bijgedragen tot het tot stand komen van dit proefschrift.

In de eerste plaatst wil ik mijn promotor Prof. Dr. Wim Vyverman bedanken om me de kans te bieden in zijn laboratorium aan onderzoek te doen. Ik kon ook rekenen op zijn enthousiasme over mijn onderzoek en op vele interessante discussies. Dr. Koen Sabbe en Dr. Koenraad Muylaert hebben me ingeweid in de manier waarop we die onooglijk kleine protistjes kunnen bestuderen. Bedankt Wim, Koen en Koenraad voor het helpen ordenen van de vele ideeën en denkpistes, voor de vele discussies en het kritisch lezen van mijn teksten. Het kostte jullie soms veel moeite om me te overtuigen, maar jullie hadden meestal weldegelijk gelijk. Al mijn directe collega's, en dat zijn Aaike, Christine, Elie, Hara, Jeroen, Katleen, Koen, Koenraad, Leen, Nele, Pieter, Sylvie, Vanessa (waar is de tijd van onze *women-talk* tijdens de middagpauzes), Victor en Wim, dank ik voor de babbels, de aanmoedigingen en de leuke sfeer op het labo. Dank ook aan de andere mensen van het laboratorium Plantkunde voor de interesse en het gezelschap.

Het Fonds Wetenschappelijk Onderzoek-Vlaanderen dank ik voor mijn mandaat als Aspirant en de reiskredieten die ze me toewezen.
Mijn onderzoek werd ook financieel ondersteund door de volgende projecten: ECOFLAT (ENV4-CT96-0216), FWO onderzoeksproject nr. G.0104.99 en GOA onderzoeksproject nr. 1205398.

Een speciale woordje van dank gaat naar Prof. Dr. Carlo Heip en de mensen van het NIOO-CEMO, voor de interessante samenwerking en het ter beschikking stellen van jullie infrastructuur (o.a. het gebruik van de Luctor) zonder dewelke mijn onderzoek niet mogelijk was geweest. In het bijzonder ook dank aan Prof. Dr. Peter Herman, Dr. Mathieu Starink, Dr. Filip Meysman, en Jan Peene die mijn experimenten op de Luctor mogelijk maakte, Pieter van Rijswijk voor de talloze regelingen ivm reservaties voor de Luctor en het gezelschap en de hulp aan boord, en de bemanning van de Luctor die steeds een warme soep klaar had na het harde werken op de plaat, en ons natuurlijk steeds veilig weer aan wal zette.

Hartelijk dank eveneens aan Prof. Dr. Magda Vincx en de overige mariene biologen van Gent, voor de samenwerking op verschillende vlakken en het ter beschikking stellen van jullie infrastructuur (o.a. de Coulter counter en het labo uitgerust voor het werken met radioactiviteit). Een speciaal woordje van dank aan Dr. Tom Moens, voor de leuke samenwerking, de inweiding in de radioactieve wereld, maar vooral bedankt Tom, voor de steun en de geruststellende praatjes. Bedankt Dirk van Gansbeke voor de pigment- and nutrientenanalyses.

Voor de vele staalnames en experimenten had ik heel wat helpende handen nodig en die werden me vriendelijk aangebonden door mijn directe collega's, maar ook door Christine van der Heyden, Danielle Schram, Ilse de Mesel, Roberto Urrutia en Sven L. Hartelijk dank allemaal!!

Chapter 1

General introduction and aims

Introduction

Estuarine systems are valuable environments, economically as a navigation route, as fishing grounds and for recreation, but also ecologically. Estuaries are very productive ecosystems (Boaden & Seed 1985, McLusky 1989). They harbour a great diversity of organisms since they are highly variable and dynamic systems with large spatial gradients (Heip et al. 1995). As they are the interface between terrestrial and marine environments, estuaries are an obligate pathway for land derived wastes on their way to the coastal zone. Both the quantity and quality of these wastes are altered during their journey through the estuary (Abril et al. 2002). Long residence time estuaries behave as efficient filters, buffering increased carbon loads due to pollution by mineralization (Abril et al. 2002). Due to high organic carbon loads, estuaries are generally highly heterotrophic systems (Heip et al. 1995).

Intertidal sediments play a crucial role in the carbon cycle of estuarine ecosystems. A considerable fraction of the organic material imported into, or produced in estuarine and coastal systems reaches the benthos, where it is either remineralized or buried (Heip et al. 1995). In meso- and macrotidal estuaries, intertidal sediments cover large areas and are an important site for accumulation and mineralization of organic matter (Heip et al. 1995). In the Westerschelde Estuary, for instance, intertidal sediments are estimated to account for about 25 % of total carbon retention (Middelburg et al. 1996). Moreover, in estuaries with large intertidal areas, the microphytobenthos in intertidal sediments may account for a considerable part of the estuarine primary production (Heip et al. 1995, Underwood & Kromkamp 1999). High amounts of allochtonous organic matter and high *in situ* primary production are the basis of an intense biological activity in intertidal estuarine sediments.

protozoan and metazoan filter feeders. Protozoa were found to be important as a food source for many zooplankton species (Stoecker & Capuzzo 1990, Arndt 1993, Sanders & Wickham 1993). The discovery of the quantitative importance of protozoa in pelagic ecosystems, their importance as bacterivores and as a prey for higher trophic levels, and the insight in the fact that a substantial fraction of the organic matter produced by microalgae is released as dissolved organic matter, were incorporated within the concept known as the microbial loop (Azam et al. 1983). The microbial loop concept drastically changed the view of the planktonic food web. In the classic planktonic food web, microalgae provided food for microzooplankton, which in turn were eaten by larger consumers. It is now known that energy released as dissolved organic matter by phytoplankton may return to the main food chain via a microbial loop of bacteria – protozoa – microzooplankton. Bacteria growing on algal exudates are consumed by flagellates, which in turn are food for the microzooplankton, mainly ciliates and rotifers. Consumption of flagellates, ciliates and other microzooplankton by mesozooplankton (e.g., copepods and cladocerans) forms a link to the traditional food chain (e.g., Stoecker & Capuzzo 1990). On the other hand, protozoa were found to enhance nutrient cycling through the excretion of nitrogen and phosphorus compounds (e.g., Goldman et al. 1985, Allali et al. 1994). In this way, they stimulate bacterial growth and accelerate organic matter decomposition (Sherr et al. 1982).

The quantitative importance of protozoa and the structure and function of their communities in intertidal estuarine sediments, and aquatic sediments in general, is poorly known. Methodological problems connected with the extraction of protozoa from the sediments and masking by sediment particles have caused a backlog in the knowledge of benthic compared to pelagic protozoa. The first quantitative studies on benthic protozoa concentrated on larger protozoa, such as ciliates and large dinoflagellates (e.g., Fenchel 1969), since these cells are most conspicuous because of their size and are most easily extracted from the sediment. The development of suitable techniques for efficient extraction of protozoa from sediments (Starink et al. 1994a, Epstein 1995), and the use of epifluorescence microscopy at high magnification, revealed high abundances of small (< 20 μm) protozoa which had previously been overlooked (Bak & Nieuwland 1989). In spite of improved methodologies, the available data on benthic protozoa remain as yet limited in terms of their temporal and spatial coverage, as well as in terms of their habitat. As far as I know, the available studies on protozoa in intertidal sediments report data on marine and a few brackish water sites only (e.g., Bak & Nieuwland 1989, Al-Rasheid & Sleigh 1995, Epstein 1997, Hamels et al. 1998, Lee & Patterson 2002), whereas data on protozoa in freshwater intertidal sediments are entirely lacking. The fact that quantitative data on benthic protozoa are still limited can partly be ascribed to the complexity of the methods used for extraction and enumeration, and the complexity of benthic ecosystems, including the compactness of environmental gradients. Likewise, the study of trophic interactions in the benthic microbial food web is more complicated than in pelagic environments because organisms live in close proximity of each other and in close association with particles. As a consequence of methodological difficulties, data on the role of protozoa as grazers of benthic resources, and as a food source for higher trophic levels are as yet very limited.

patterns in the ciliate species composition and the abundances, and to identify the factors potentially regulating the ciliate community dynamics in these sediments.

The remaining chapters focussed on **the role of protozoa as grazers and as a potential food source for higher trophic levels.**

Chapter 4 focussed on the potential role of heterotrophic flagellates as consumers of bacterial production in estuarine sediments. Due to high amounts of organic carbon, high bacterial abundances and production rates have been measured in intertidal estuarine sediments (e.g., van Duyl & Kop 1990, Wellsbury et al. 1996). However, in contrast to planktonic ecosystems, the fate of bacterial production in aquatic sediments is still largely unclear. Grazing studied with benthic flagellates are scarce, and mostly reveal only a small impact of flagellate grazing on benthic bacterial production (e.g., Starink et al. 1996). Nevertheless, it has been suggested that grazing rate estimates have probably been underestimated because grazing on attached bacteria was neglected (Starink et al. 1994b). The impact of flagellate grazing on benthic bacterial production was studied at a sandy and a silty site on the Molenplaat, at 5 occasion during the course of 1 year. Grazing rates of heterotrophic flagellates on free and attached bacteria in the sediments were estimated using fluorescently stained sediment. Simultaneously with the grazing estimates, bacterial production was measured using ^{3}H-leucine incorporation. Comparison of total flagellate grazing and the bacterial carbon production provided an estimate of the impact of flagellate bacterivory on bacterial production.

Intertidal estuarine sediments, including Schelde sediments, often sustain very dense diatom populations (Sabbe 1993, Underwood & Kromkamp 1999). These diatoms are important primary producers and an important food source in estuaries. Although many protozoans feed on algae, only few investigations have considered the influence of benthic protozoan herbivory. Observational and experimental studies have shown that phagotrophic ciliates are highly selective predators. However, the available data on selective feeding by benthic algivorous ciliates are derived from the analysis of food vacuole contents, which are compared to the composition of the available diatom species (e.g., Balczon & Pratt 1995). This approach gives only limited insight in the actual mechanisms involved in prey selection and the relative importance of passive selection, governed by the relative availability and vulnerability of the prey items, and active or behavioral selection. In the present work, the mechanism of prey selection in benthic ciliates feeding on mixed diatom assemblages was studied using direct behavioral observations (**Chapter 5**). The feeding preferences of 4 ciliates species were established, as well as relative encounter rates, attack probabilities and capture successes in various 2-species diatom mixtures. The influence of prey ratio, prey abundance and feeding history was also determined. As the behavioral observations strongly suggested that selective encounters with the diatoms were caused by non-contact detection of individual prey items, an additional experiment was set up using T-mazes and aimed to test whether the ciliates were able to distinguish between diatom species on the basis of soluble chemical cues.

In contrast to pelagic ecosystems, little is known about links between protozoa and higher trophic levels in sediments. Nematodes are numerically the dominant metazoans in most intertidal estuarine sediments. Although some nematode taxa were shown to be capable of ingesting ciliates,

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Chapter 2

Quantitative importance, composition and role of protozoan communities in polyhaline and freshwater estuarine intertidal sediments

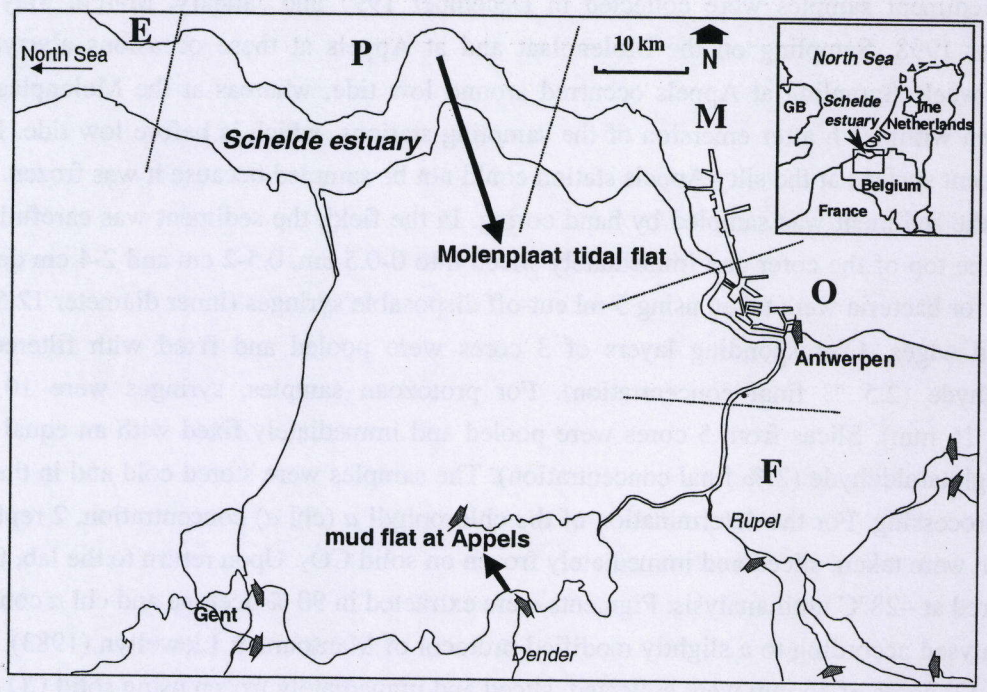
Ilse Hamels, Koen Sabbe, Koenraad Muylaert & Wim Vyverman

Manuscript in preparation

Abstract

The present study investigated the quantitative importance of protozoa in intertidal sediments of a polyhaline and a freshwater site in the Schelde estuary and tried to evaluate the potential role of protozoa in these sediments. Protozoan abundances and biomasses were determined at a sandy and a silty station at either site, bimonthly during the course of 1 year, and at 3 sediment depths. Ciliates and dinoflagellates were identified up to the species level where possible, whereas other protozoa ≤ 20 μm were classified as nano-heterotrophs. Total biomass of the protozoans studied, integrated over the upper 4 cm of the sediment, was in the same order of magnitude at the polyhaline and the freshwater intertidal site, and ranged from 41 to 597 mg C m^{-2}. Nano-heterotrophs were the dominant protozoans at the 4 sampling stations, both in terms of abundance and biomass. Differences in their quantitative importance in the polyhaline compared to the freshwater intertidal sediments were small. Ciliates were more abundant in the polyhaline reaches, and differed strongly in their species composition between the sites. These differences can be explained by differences in sediment characteristics and hydrodynamical disturbances between the sites. Heterotrophic dinoflagellates were almost exclusively found at the sandy station in the polyhaline reaches of the estuary. Biomass ratios of bacteria and algae to protozoa suggest that protozoa have a higher grazing impact at the sandy compared to the silty station at both sites, and at the polyhaline compared to the freshwater intertidal site. Protozoan and metazoan biomasses at our sampling stations in late spring/early autumn, and estimates of their weight-

Fig. 1. Schelde estuary, with the location of the mud flat at Appels in the freshwater tidal part of the estuary and the Molenplaat intertidal flat in the polyhaline reaches. The borders between the salinity zones are indicated by a dotted line (E: euhaline, P: polyhaline, M: mesohaline, O: oligohaline, F: freshwater tidal). The upper limit of tidal influence is indicated with grey arrows



Materials and methods

Study site and sampling

The sampling stations were located in the intertidal zone at 2 sites in the Schelde estuary. The first sampling location, a mud flat at Appels near Dendermonde, is situated in the freshwater tidal reaches of the Schelde estuary (salinity < 0.5, Zeeschelde, Belgium; Fig. 1), about 125 km from the mouth of the estuary. The tidal amplitude is about 3.4 m at Appels. High sedimentation rates and high concentrations of suspended particulate matter (SPM) have been found at this location (Criel 1999, Muylaert et al. in press). Moreover, suspended sediments have a high clay content at Appels (~45 to 90 %; Wartel & Francken 1999). High mineralization rates cause oxygen depletion in summer in the freshwater tidal reaches of the Schelde estuary. The other sampling location, *viz.* the Molenplaat intertidal flat, is located in the upstream part of the polyhaline reaches of the estuary (salinity 18 to 30, Westerschelde, SW Netherlands; Fig. 1), about 35 km from the mouth of the estuary. This intertidal flat is subject to a tidal amplitude of about 5 m. The brackish and marine parts of the estuary are largely influenced by the tidal input of relatively clean marine sediments and oxygen-rich seawater. At both sites, 2 stations with contrasting characteristics were selected. For convenience, and given the differences in sediment characteristics of these stations, they will be called the sandy and silty stations. At the Molenplaat, the silty station is located in the central, most protected region of the flat, while the sandy station is situated near the edge of the flat and is subject to higher hydrodynamical disturbances. The emersion period for both stations is about 7 h. At Appels, the silty station is situated near the mean high tide line (emersion time ~10 h) and the sandy station near the

1993, with small modifications), all ciliates and dinoflagellates on these filters were enumerated and identified (see Chapter 3 for the ciliates; using Larsen 1985 for the dinoflagellates). Another subsample of the Percoll supernatant was stained with DAPI (5 μg ml^{-1} final concentration) and collected on a 1 μm Nuclepore polycarbonate filter. Filters were mounted on slides in immersion oil and stored frozen in the dark until enumeration, which took place within 2 months. On these filters, protozoa other than ciliates and dinoflagellates (both were easily recognizable by their shape and their nuclei) were counted using epifluorescence microscopy with UV excitation. They were classified by their longest linear dimension into 3 different size classes: < 5 μm, 5-10 μm, 10-20 μm. Absence of chlorophyll was checked by switching to blue light excitation. On the DAPI stained filters, the presence or absence of pigments was also checked for the dinoflagellate species that were counted together with the ciliates. Cells with irregular, amoeboid shapes were sometimes very abundant. A disadvantage of epifluorescence microscopy, which is frequently used for flagellate counts, is that the presence of flagella may be difficult to resolve. Epifluorescence counts may include for example small naked amoebae, yeasts, zoospores etc. (Arndt et al. 2000). Because of the uncertain taxonomic composition of the cells counted using epifluorescence microscopy, the term nano-heterotrophs was used. Cells with irregular, amoeboid shapes were distinguished, resulting in the categories 'regular nano-heterotrophs' and 'amoeboid nano-heterotrophs'. Per filter, at least 100 randomly selected fields were observed (magnification 1000x).

For ciliates, dinoflagellates and nano-heterotrophs, biovolumes were estimated assuming cells to have simple geometrical shapes and were converted to carbon biomass assuming a conversion factor of 200 fg C μm^{-3} (Børsheim & Bratbak 1987, Sherr & Sherr 1993) for aldehyde fixed protozoa.

Statistical analysis

Differences between the 4 sampling stations for the abiotic and biotic factors measured were tested using 1-way ANOVA for repeated measures. The Student-Newman-Keuls multiple comparison test was used for *post hoc* pairwise comparisons. Statistical analyses were performed with STATISTICA 5.1 for Windows (StatSoft Inc., Tulsa, OK, USA). Data were log $(x + 1)$ transformed to meet the assumptions of normality and homogeneity of variances.

Results

Abiotic factors

At Appels, the sediment at the silty station consisted of 70 to 94 % mud (fraction < 63 μm) and the median grain size (mgs) averaged 17 ± 4 μm (Fig. 2A, B). The mgs at sandy Appels station was significantly higher and averaged 162 ± 17 μm (Fig. 2A; Table 1). The mgs was slightly, but not significantly, higher at the sandy Molenplaat station compared to the sandy Appels stations, but the mud content at these stations, was significantly lower at the Molenplaat (Fig. 2A, B; Table 1). At the silty Molenplaat station, the mgs averaged 108 ± 20 μm, which is considerably higher compared to the silty Appels station (Fig. 2A). In general, vertical (not shown) and seasonal (Fig. 2A, B) fluctuations in the grain size distribution were small at the 4 stations. Nitrate concentrations in the interstitial water were significantly higher at the Molenplaat than at Appels (Fig. 2C; Table 1).

Table 1. Results of Student-Newman-Keuls tests for differences in the abiotic and biotic factors measured. Underlined groups are not significantly different ($p > 0.05$); sampling stations are in ascending order. The values used for the tests are the average values, weighted over the 3 depth layers, for each of the sampling occasions (for abiotic factors), or biomasses integrated over 4 cm sediment depth (for biotic factors). Protozoa: nano-heterotrophs + ciliates + heterotrophic dinoflagellates; A: Appels; MP: Molenplaat

mgs	A-silty < MP-silty < A-sandy MP-sandy
mud content	MP-sandy < A-sandy < MP-silty < A-silty
nitrate	A-sandy A-silty < MP-silty < MP-sandy
ammonium	MP-sandy < A-silty MP-silty A-sandy
chl *a*	MP-sandy < A-sandy MP-silty < A-silty
bacteria	MP-sandy < A-sandy MP-silty < A-silty
nano-heterotrophs	A-sandy MP-silty A-silty MP-sandy
ciliates	A-sandy A-silty MP-silty < MP-sandy
protozoa	A-sandy MP-silty A-silty < MP-sandy

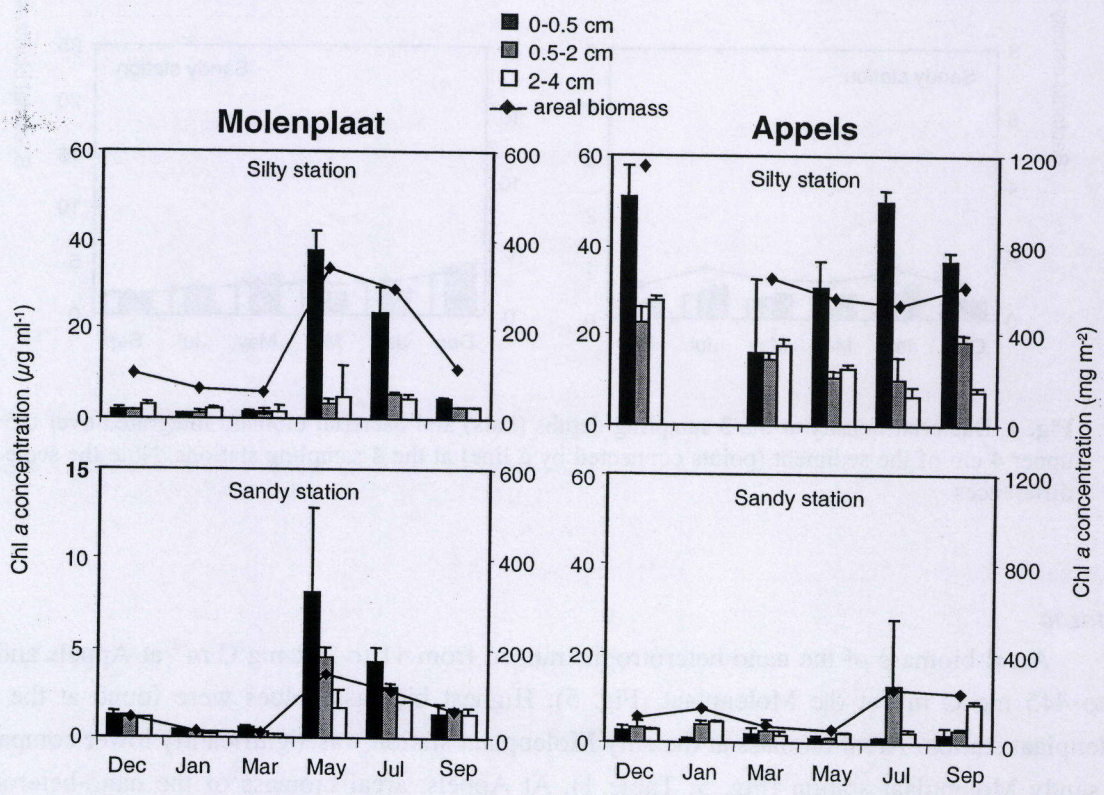


Fig. 3. Chl *a* concentration at the 3 sampling depths (bars; mean ± 1 SD) and integrated over the upper 4 cm of the sediment (points connected by a line) at the 4 sampling stations. For clarity, SD is omitted for the areal values. Note the scale differences

but there was a decrease of the abundances with sediment depth. Moreover, 48 to 70 % of the nano-heterotrophs at the sediment surface of this station were > 5 μm in size, whereas beneath this surface layer, cells were usually smaller (Fig. 5). These differences are reflected in the biomass values, which averaged 14.9 ± 7.6 μg C ml^{-1} at the sediment surface and 2.9 ± 3.1 μg C ml^{-1} in subsurface sediments at the silty Appels station (not shown). A general vertical pattern was not found at the sandy station at Appels (Fig. 5). At the latter station, nano-heterotrophic abundances averaged 8.5 ± 3.6 x 10^4 cells ml^{-1} (Fig. 5), corresponding to 2.6 ± 2.1 μg C ml^{-1} (not shown), and 49 to 87 % of the cells were < 5 μm at every depth. At the sandy Molenplaat station, nano-heterotrophic abundance and biomass averaged 8.4 ± 3.7 x 10^4 cells ml^{-1} (Fig. 5) and 7.8 ± 4.2 μg C ml^{-1} (not shown), and 45 to 79 % of the nano-heterotrophs were > 5 μm in size (Fig. 5). With a few exceptions, densities of nano-heterotrophs were generally lower (on average 5.6 ± 4.9 x 10^4 cells ml^{-1}) and cells were usually smaller at the silty compared to the sandy Molenplaat station (Fig. 5). A considerable fraction of the nano-heterotrophs was amoeboid at Appels, especially at the silty station (Fig. 5). In December and September, at the silty Appels station, 73 % of the nano-hetertrophs at the sediment surface was amoeboid (abundance up to 1.9 x 10^5 cells ml^{-1}). At the sandy Appels station, amoeboid cells were always more abundant below the sediment surface and reached densities up to 5 x 10^4 cells ml^{-1}. At the Molenplaat, amoeboid nano-heterotrophs were nearly absent at the sandy site, whereas only a small fraction of the nano-heterotrophs at the silty site was amoeboid on some occasions (maximum 5.8 x 10^3 cells ml^{-1}).

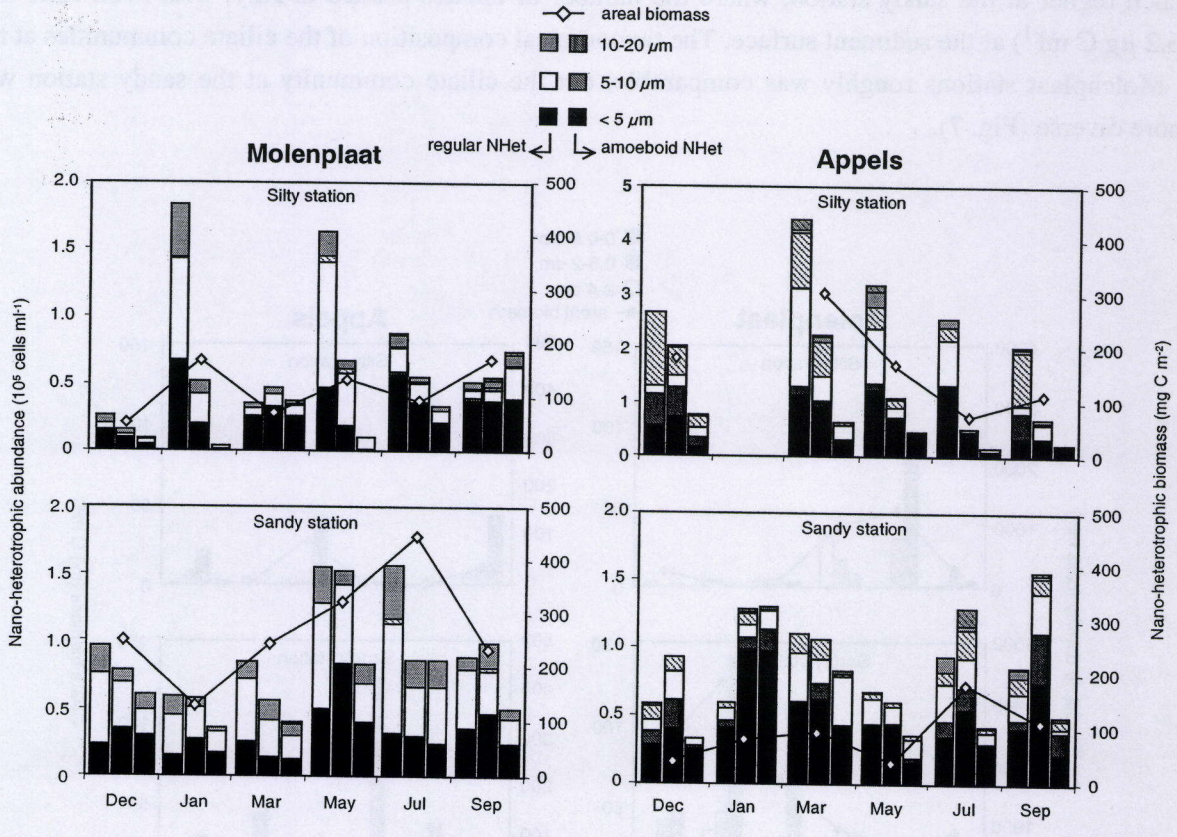


Fig. 5. Nano-heterotrophic abundance at the 3 sampling depths (bars) and nano-heterotrophic biomass integrated over the upper 4 cm of the sediment (points connected by a line) at the 4 sampling stations. Total density is subdivided proportional to the contribution of the different size classes, and the contribution of regular (not hatched) and amoeboid (hatched) cells (see text). Note the scale differences. NHet: nano-heterotrophs

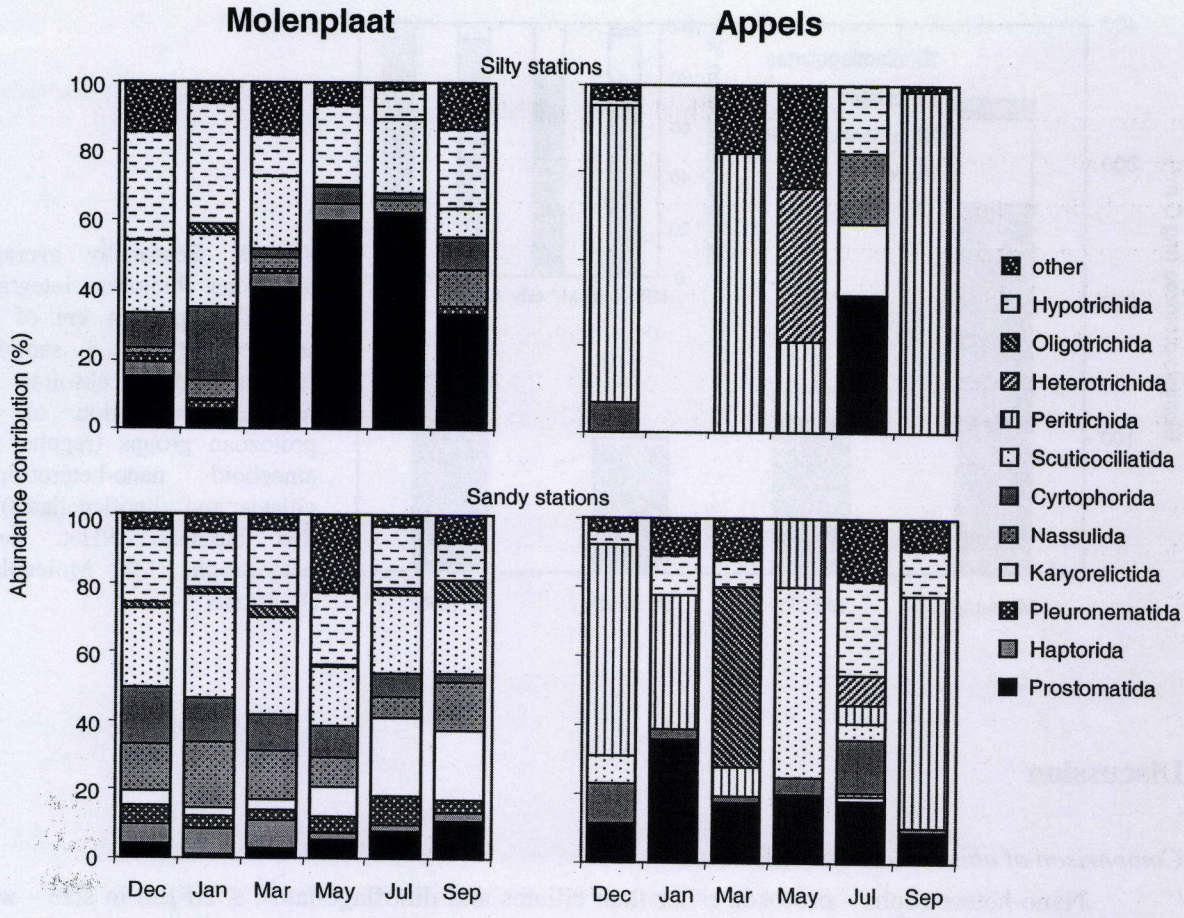


Fig. 7. Taxonomical composition of the ciliate communities at the 4 sampling stations. Relative contribution of the most common orders to ciliate abundance integrated over the upper 4 cm of the sediment

Heterotrophic dinoflagellates were absent at Appels, whereas at the Molenplaat, they were mainly found at the sandy station. At the sandy Molenplaat station, highest densities were found at the sediment surface in May, July and September (up to 2.2 x 10^3 cells ml^{-1}; not shown). The dominant taxa were *Amphidinium semilunatum* Herdmani and *Amphidinium scissum* Kofoid & Swezy. Biomass ranged from 8.3 to 26.3 mg C m^{-2} at the sandy Molenplaat station and was always ≤ 0.5 mg C m^{-2} at the silty Molenplaat station (not shown).

Total biomass of all studied protozoans integrated over the upper 4 cm of the sediment was in the same order of magnitude at Appels and the Molenplaat: 41 to 301 mg C m^{-2} and 61 to 597 mg C m^{-2}, respectively. Areal biomass of the protozoa was significantly higher at the sandy Molenplaat station compared to the other 3 stations (Table 1). Nano-heterotrophs always by far dominated total protozoan abundance and biomass at the 4 sampling stations. Seasonally averaged, they accounted for between 77.8 and 94.6 % of areal protozoan biomass (Fig. 8). At Appels, on average one third of these nano-heterotrophs was amoeboid. At the Molenplaat, amoeboid cells accounted for only on average 5 and 0.8 % of areal biomass at the silty and the sandy station, respectively (Fig. 8). Ciliates accounted for 5.4 to 17.8 % of the average areal protozoan biomass. Heterotrophic dinoflagellates accounted for up to 7 % of areal protozoan biomass at the sandy Molenplaat station (Fig. 8).

do not always allow to distinguish between flagellates and other unicellular organisms such as amoebae, the terms 'nano-heterotrophs' in the present study and the term 'nanoflagellates' in the above mentioned studies comprise more or less the same group of organisms. Data obtained using other counting techniques (e.g., live counts on diluted sediment) are usually lower than epifluorescence counts (e.g., Alongi 1986, Gasol 1993), presumably because the smallest protozoa were underestimated. Ciliate abundances ranged from 0 to 3.8 x 10^3 cells ml^{-1} in the present study. These values fall within the very wide range of ciliate abundances found in marine and freshwater sediments (from below the detection limit to > 10^4 cells ml^{-1}; e.g., Hansen & Alongi 1991, Gasol 1993, Packroff & Zwick 1998, Garstecki et al. 2000). Heterotrophic dinoflagellates were almost exclusively found at the sandy Molenplaat station, which seems to agree with literature data that report benthic dinoflagellates almost exclusively from marine sands (Fenchel 1967, 1969, 1987, Alongi 1986, Patterson et al. 1989, Fernandez-Leborans & Novillo 1992, Lee & Patterson 2002). The abundance of heterotrophic dinoflagellates amounted to 2.2 x 10^3 cells ml^{-1} in the present study. Although quantitative data on dinoflagellates are scarce, dinoflagellate abundances up to > 1 x 10^3 cells cm^{-2} (Fenchel 1967, 1969) and > 1 x 10^4 cells ml^{-1} (Lee & Patterson 2002) have been found, but comprise all dinoflagellates, i.e., heterotrophic as well as pigmented ones. Pigmented dinoflagellates were also abundant at the sandy Molenplaat station (viz. *Amphidinium herdmanii* Kofoid & Swezy) and reached abundances up to 7 x 10^3 cells ml^{-1} (not shown).

Appels versus the Molenplaat

Since, to our knowledge, data on protozoa in freshwater intertidal sediments are entirely lacking, protozoan communities in saline and freshwater intertidal sediments of an estuary have never been compared. One might expect an intense biological activity in freshwater intertidal sediments, since nutrient concentrations are high and sediments are richer in organic carbon than in brackish and marine estuarine reaches. Although there was some overlap between the values, bacterial and algal biomasses were in general higher at Appels than at the Molenplaat. Nevertheless, the present study revealed that total areal biomass of the protozoa studied was in the same order of magnitude in the polyhaline sediments of the Molenplaat and the freshwater sediments at Appels. In spite of this, some differences between the sites were found for single protozoan groups, especially for the ciliates.

The most obvious difference between the ciliate communities at Appels and the Molenplaat is the fact they showed few taxonomical similarities. Moreover, ciliates were generally less abundant at Appels (max. 0.3 x 10^3 ciliates cm^{-2}) compared to the Molenplaat, where the abundance at the 2 stations was > 2.5 x 10^3 ciliates cm^{-2} for at least part of the year. Interstitial space is considered as one of the main constraining factors for ciliates in sediments (Fenchel 1969, Patterson et al. 1989). In marine sediments, where the ecology of ciliates has been studied in more detail, fine sandy sediments (120-250 μm) are generally found to harbour the most diverse and abundant ciliate communities (e.g., Fenchel 1969, Epstein 1997a, Hamels et al. 1998). Our observations at the Molenplaat are in accordance with these data, since ciliate abundances were higher at the sandy compared to the silty station. Ciliate abundances in sandy sediments at Appels, on the other hand, were much lower when compared to the sandy sediments at the Molenplaat. Although the mgs at these sites did not differ significantly, the mud content was higher at Appels (on average 12 ± 3.9 % versus 2.5 ± 0.8 %). This means that the size of the individual pores, which is important to the ciliates, was smaller at the sandy Appels stations, since interstitial space is very sensitive to sorting and is drastically reduced in

the nano-heterotrophs differed between Appels and the Molenplaat. We cannot exclude a taxon-specific reaction to fixation or staining, but to our knowledge such a response has never been observed. Otherwise, the amoeboid cells might be amoebae, as suggested by their shapes. According to Fenchel (1987), naked amoebae are of similar importance in freshwater and marine ecosystems. On the other hand, naked amoebae are generally more abundant in silty compared to sandy sediments, and are able to live and reach high abundances in very muddy sediment (Butler & Rogerson 1995, 1996, Anderson 1998). Although the available data are still contradictory (e.g., Alongi 1990, Hondeveld et al. 1994, Hamels et al. 1998), flagellate abundances in sediments are mostly higher in fine sandy than silty and muddy sediments (e.g., Bak et al. 1991, van Duyl et al. 1992, Bak & Nieuwland 1993). Unlike ciliates and most flagellates, amoebae live in association with surfaces and need no interstitial spaces for locomotion or feeding. Therefore, higher numbers of naked amoebae at Appels, especially at the silty station, are not unlikely. Supposing the amoeboid nano-heterotrophs were naked amoebae, then their abundance at the silty Appels station (up to 1.9 x 10^5 cells ml^{-1}) was high compared to the abundances found in other sediments (generally ~10^3 to 10^4 cells ml^{-1}; Butler & Rogerson 1995, Decamp et al. 1999, Garstecki & Arndt 2000, Garstecki et al. 2000).

Protozoa and other benthic organisms

Little is known about the role of protozoa in intertidal estuarine sediments, and aquatic sediments in general. In pelagic ecosystems protozoa have been identified as major consumers of bacteria and phytoplankton (Sherr & Sherr 1994). The ratio of bacterial and algal biomass to the biomass of protozoa gives an idea about the potential influence of protozoa on the standing stock of the available carbon sources at our sampling station. The relation of bacterial biomass to the biomass of protozoa was lower at the sandy compared to the silty station at Appels as well as on the Molenplaat (Table 2). However, this ratio was comparatively higher at the Appels stations. The same holds true for the ratio of algal (chl *a*) to protozoan biomass (Table 2). Higher ratios at Appels are mainly the result of high bacterial and algal biomasses at this site.

Table 2. Average ratio of bacterial and algal biomass to protozoan biomass. The values used are areal carbon biomass values. Algal carbon biomass was estimated from chl *a* concentrations assuming a carbon to chl *a* ratio of 40 (de Jonge 1980)

	bacteria/ protozoa	algae/ protozoa
Appels silty	104	187
Appels sandy	42	62
Molenplaat silty	27	58
Molenplaat sandy	2.6	6.5

These ratios suggest that protozoa have the highest grazing impact at the sandy Molenplaat station. At this station, the biomass of protozoa is, on average, only ~2.6 times smaller than bacterial biomass. It has been estimated that up to more than 60 % of bacterial production can be consumed by bacterivorous nano-heterotrophs and ciliates at the sandy Molenplaat station (Hamels et al. 2001b).

benthic energetics is disproportional to their biomass. Weight-specific metabolic rate (R) is related to body weight (W) according to $R = a\ W^{-0.25}$ (Fenchel 1974). On the basis of this formula, and an estimated mean individual body weight (roughly total biomass/total abundance) and biomass for each group, relative metabolic rates were calculated for ciliates, nano-heterotrophs and metazoa at each of our sampling stations (Table 3). These ratios are, of course, nothing more than rough estimates but they clearly suggest that, relative to the metazoa, protozoa are important components in sediment respiration, especially at the sandy stations. We find that the relative importance of protozoa in late spring/early autumn (see Table 3) is ~29 to 96 % when the combined metabolic rate of benthic consumers at our sampling stations is considered. In general, ciliates were the least important group at the 4 stations, but the metabolism of nano-heterotrophs exceeded meio- and macrobenthic metabolism at the sandy Molenplaat station and oligochaet metabolism at the sandy Appels station (Table 3). The data emphasize the importance of small protozoa in sediments and suggest that protozoa should be recognized as a full member of benthic ecosystems.

Acknowledgements

This research was performed in the frameworks of the EU Environment & Climate programme ECOFLAT (ENV4-CT96-0216), which is part of the ELOISE programme, the FWO research project no. G.0104.99 and the GOA research project no. 1205398. I. H. acknowledges a grant from the Fund for Scientific Research-Flanders (FWO), K. S. and K. M. are postdoctoral fellows of the same Fund. Thanks to Dirk van Gansbeke for chlorophyll and nutrient analyses. We also thank the Netherlands Institute of Ecology-Centre for Estuarine and Coastal Ecology (NIOO-CEMO) for the use of the RV 'Luctor'. Prof. P. M. J. Herman, M. Steyaert, and Drs. C. Barranguet and J. Seys kindly provided data on the biota and the oxygen conditions at the sampling stations.

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Chapter 3

Contrasting dynamics of ciliate communities in sandy and silty sediments of an estuarine intertidal flat

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Manuscript in preparation

Abstract

During a 1-year study of the ciliate fauna of a silty and a sandy site on an intertidal flat in the Westerschelde estuary a total number of 107 taxa were recorded belonging to at least 52 genera and 15 orders. Total ciliate abundance ranged from 0.2 to 5.6 x 10^3 cells cm^{-2} integrated over the upper 4 cm of the sediment. At the silty site, ciliate abundance decreased strongly with the onset of siltation after winter. At this site, the rich winter assemblage of ciliates at the sediment surface disappeared towards summer, whereas the ciliate fauna in subsurface sediments was poor but changed little throughout the year. Species richness of the ciliate community and ciliate abundance were higher at the sandy site. Moreover, seasonal and vertical dynamics were less pronounced at this site. Ciliate abundances at the sandy site changed gradually from a winter minimum to a maximum in summer. Simultaneously, the vertical distribution pattern of the ciliates shifted upwards. These results show that sediment characteristics were an important factor regulating the ciliate communities at the Molenplaat. The median grain size of the sediment was the most important predictor of ciliate abundance according to a multiple regression analysis. Moreover, multivariate analyses indicated strong differences in species composition between samples with a high mgs on the one hand and samples with a high mud content on the other hand. The differences between the sandy and the silty site, and seasonal patterns at the silty site demonstrate that physical properties of the sediment were more important for the ciliates than food availability or temperature. The results from the sandy site suggest that temperature and the availability of food and

Methods

Study site and sampling

This study was carried out at the Molenplaat, an intertidal flat in the polyhaline reaches of the Schelde estuary (SW Netherlands). Salinity in this region of the estuary is about 20 to 25. The 2 sampling sites chosen (Fig. 1) differ with respect to sediment characteristics and were studied in the framework of the ECOFLAT (Eco-metabolism of an estuarine tidal flat) project (for a description of the project and a full site description, see Herman et al. 2001). The silty site (Molenplaat station 2) has a central and protected location on the flat and experiences accumulation of mud during summer. In late spring and summer, a well-developed diatom mat is found at this site, which greatly enhances sediment stability and prevents resuspension of the sediment (Lucas et al. 2000). The sandy site (Molenplaat station 4), on the other hand, is located towards the edge of the flat and is characterized by a relatively high bottom shear stress (1.15 Pa compared to 0.36 Pa at the silty site; Herman et al. 2001). Strong physical and biological mixing at this site prevent accumulation of fine materials as well as the development of a dense algal mat. Organic carbon content and bacterial production are 1 order of magnitude higher at the silty site than at the sandy site, while primary production is comparable at both sites (Hamels et al. 2001b, Herman et al. 2001).

Samples were collected during ebb tide in December 1997 and January, March, May, July and September 1998. The upper 4 cm of the sediment was sampled by hand coring using 10 ml cut-off disposable syringes (inner diameter 16 mm) with sharpened edges. In the field, the sediment was carefully extruded through the top of the cores and immediately sliced into 0 to 0.5 cm, 0.5 to 2 cm and 2 to 4 cm depth layers. Corresponding layers of 5 replicate cores, collected within a surface area of 0.5 m^2, were pooled and fixed with an equal volume of ice-cold glutaraldehyde (2 % final concentration).

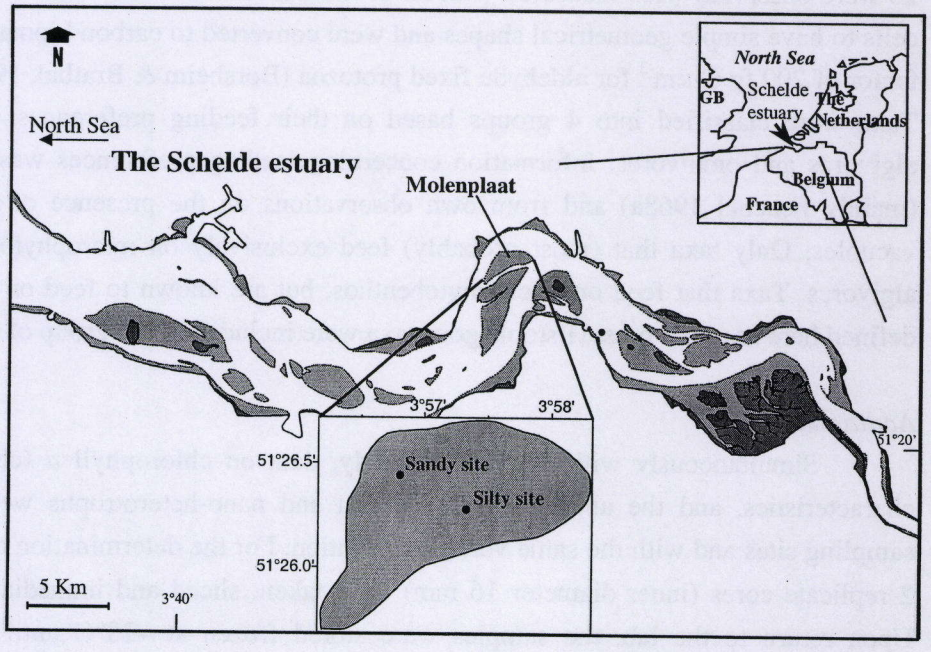

Fig. 1. Schelde estuary, with the location of the sampling sites on the Molenplaat tidal flat

sliced, immediately frozen using solid CO_2 and stored frozen at –28°C. From the thawed sediment, interstitial water was extruded at high pressure for the analysis of nitrate-N and ammonium-N concentrations. These analyses were performed with a Skalar auto-analyser, according to the continuous segmented flow principle (Greenberg et al. 1992). The ratio of nitrate to ammonium is informative on the availability of free oxygen. Since ammonium is converted to nitrate in the presence of oxygen, ammonium concentrations decrease and nitrate concentration increase when oxygen concentrations increase. The remaining sediment was used for the determination of the sediment grain size distribution using laser diffraction with a Coulter® LS 100 with fluid module (Coulter Electronics, Inc.).

Samples for bacteria were taken with 5 ml syringes (inner diameter 12.5 mm). Corresponding layers from 3 replicate cores were pooled and fixed with filter-sterilized formaldehyde (2.5 % final concentration). Bacteria were dislodged from the sediment, stained with acridine orange and counted using epifluorescence microscopy as described in Hamels et al. (2001b). Bacterial biomass was estimated assuming a mean bacterial volume of 0.14 μm^3 per cell (mean value based on Cammen & Walker 1986, van Duyl en Kop 1990, Kuwae & Hosokawa 1999) and a conversion factor of 220 fg C μm^{-3} (Bratbak & Dundas 1984).

Heterotrophic protists other than ciliates (such as flagellates) are also extracted from the sediment using density gradient centrifugation with Percoll (Starink et al. 1994). Therefore, after centrifugation in Percoll (see above), another subsample of the diluted supernatant was filtered over a 1 μm Nuclepore polycarbonate filter and stained with DAPI (5 $\mu g\ ml^{-1}$). Filters were mounted on slides in immersion oil, and stored frozen in the dark until enumeration within 2 months. Small (< 20 μm) heterotrophic protists other than ciliates were counted using epifluorescence microscopy with UV excitation. Absence of chlorophyll was checked by switching to blue light excitation. Since the taxonomic identity of cells counted using epifluorescence microscopy is uncertain (Arndt et al. 2000), the term nano-heterotrophs was used. The cells were classified by their longest linear dimension into 3 different size classes: < 5 μm, 5 - 10 μm, 10 - 20 μm. Per filter, at least 100 randomly selected fields were observed (magnification 1000x). Biovolumes were estimated and converted to carbon biomass as for the ciliates (see above).

Water temperature data were obtained from the National Institute for Coastal and Marine Management/RIKZ (the Netherlands).

Data analysis

Pearson correlation analyses were used to estimate the relationship between ciliate abundance and biotic and abiotic factors. The software package STATISTICA 5.1 for Windows (StatSoft Inc., Tulsa, OK, USA) was used for the analyses. If necessary to obtain normal distribution of the data, values were log (x + 1) transformed; percentage data underwent an arcsinus square root transformation. For correlations with water temperature data, areal ciliate abundances were used.

Multivariate indirect ordination techniques were used to visualize differences in the species composition among samples. Analyses were performed with abundance data from the samples from both sites, as well as on the data from each site separately. Environmental factors were used as supplementary or passive variables (ter Braak & Smilauer 1998). Data were log (x + 1) transformed prior to the analyses; percentage data underwent an arcsinus square root transformation. Temperature data were not used for these analyses, since, in contrast to the other factors, this factor was not

(Fig. 2). In early winter and from late spring onwards ciliate abundances at the silty site were low at every sediment depth, averaging 69 ± 56 ciliates ml^{-1}. Biomass displayed the same patterns with respect to seasonal and vertical distribution as the abundance data. Ciliate biomass integrated over the upper 4 cm of the sediment ranged from 0.16 to 4.8 μg C cm^{-2} (on average 0.7 ± 1.5 μg C ml^{-1}) at the silty site and from 2.4 to 12.6 μg C cm^{-2} (on average 1.8 ± 1.5 μg C ml^{-1}) at the sandy site (not shown).

Table 1. Total ciliate abundance and the number of ciliate taxa per sample and per sampling occasion, and the occurrence of the ciliate taxa of which the abundance exceeded 15 cells ml^{-1} in at least 1 sample. The size of the circles represents ciliate abundance as follows: (•): 15-49, (•): 50-149, (•): 150-299 and (•): ≥ 300 ciliates ml^{-1}. 1: sediment surface, 2: 0.5-2 depth layer, 3: 2-4 cm depth layer. The food preference is depicted as follows: A: algivorous, B: bacterivorous, C: carnivorous, O: omnivorous. ?: unknown

Taxa	Order	Food	Silty site: Dec. 1 2 3	Jan. 1 2 3	March 1 2 3	May 1 2 3	July 1 2 3	Sept. 1 2 3	Sandy site: Dec. 1 2 3	Jan. 1 2 3	March 1 2 3	May 1 2 3	July 1 2 3	Sept. 1 2 3
Stephanopogon colpoda		O	•	•					• • •	•	• •	• • •	• • •	•
Tracheloraphis longicollis	Karyorelictida	C							• • •	• • •	• •	• • •	• • •	
Tracheloraphis sp.	"	C							•	•	• •	• • •	• • •	• • •
Remanella sp.	"	C							• • •	•	• •	• • •	• • •	• • •
Cryptopharynx setigerus	"	O			•				• • •	•	• • •	• • •	• • •	• • •
Metacystis truncata	Prostomatida	O	• •	• •	• • •	•	• •	•				•		• • •
Metacystis striata	"	O			• • •	• • •	• •		•	• •	• •	• •	• • •	• • •
Prorodon sp.	"	O	•		•			•	•	•	• •	• •	• • •	• • •
Coleps sp.	"	O									•	• •	• • •	• • •
sp. 1	"	B	•	• •	• •	• •	• •	•	• •		•	• •	• •	• • •
sp. 2	"	?		•	• •			•						• •
Lacrymaria caudata	Haptorida	C							• •	• • •	•	•	•	•
Lacrymaria coronata	"	C	•	•	• •	•			• •	• • •	• •	•	•	• •
Lacrymaria sp.	"	C												•
Mesodinium pulex	"	B							• • •	• • •	•	•	•	
Loxophyllum uninucleatum	Pleurostomatida	C							• • •	• • •	•	• •	• •	• •
Loxophyllum verrucosum	"	C	•	•	• • •			•	• • •	•	• • •	• • •	• • •	• • •
Loxophyllum sp. 1	"	C							•	•		• •	• • •	• •
Loxophyllum sp. 2	"	C		• •	• •				• • •	• • •	• •	• •	• • •	• •
Lopezoterenia torpens	Nassulida	B					•	•	• • •	• • •	• •	• •	• •	• • •
Discotricha papillifera	"	A	•	• •	• •		•	•	• • •	• • •	• •	• •	• •	• • •
Thigmogaster pardus	Cyrtophorida	B	•	•	• •				• • •	• • •	• • •	• •	• • •	• • •
Chlamydodon triquetrus	"	A	•	•		•		•				•	•	•
Atopochilodon distichum	"	O		• •					•	•		•	•	
Chlamydonella pseudochilodon	"	A		• •	•	•			•	• •	•	•	•	• •
Chlamydonella sp. 1	"	O			• •				•	•		•	• •	
Chlamydonella sp. 2	"	A	•			•			• •	• • •	• •	•	•	•
Lynchella nordica	"	A										•	•	
Mirodysteria decora	"	B								•		• •	•	•
Dysteria pusilla	"	B	• •	• •	• •		•		• •	•		• •	• • •	
Helicostoma notatum	Scuticociliatida	C	•	• •	•					•		•		
Uronema sp.	"	B		•	•							•		
Cohnilembus stichotricha	"	B		• • •	• • •		• •							
Pseudoplatynematum loricatum	"	B							•			•		
Sathrophilus marinum	"	B							•	• •		• •	• •	• • •
Pleuronema sp. 1	"	B							• •	• • •	• •	• •	• •	• •
Pleuronema sp. 2	"	B		•					•			•		•
Hippocomos loricatus	"	O		•										
Cyclidium plouneouri	"	B		• •	• •		• •		• • •	• • •	• • •	• • •	• • •	• • •
Cyclidium sp.	"	B										• •	•	•
Histiobalantium marinum	"	O							• •	•		•	•	• •
sp. 3	"	O	•	• •			•		• •	•	• •	• •	•	• •
Peritromus arenicolus	Heterotrichida	O									•	•	•	•
Strombidium arenicola	Oligotrichida	A									• •	•		•
Strombidium sp.	"	?		• •										
Stobilidium sp.	"	A		•						• •				
Aspidisca fusca	Hypotrichida	B	• •	• •	• • •				• • •	• • •	• • •	• • •	• • •	• • •
Aspidisca major	"	B	• •	• •	• •				• •	• •	•	• •	•	•
Aspidisca pulchirrima	"	B		• •					•	•		•		
Aspidisca steini	"	B	• •	• •	• •				• •	•	• •	• •	• •	•
Aspidisca sp. 1	"	B	• •	•	• • •	• • •	• •	• • •						
Aspidisca sp. 2	"	O	•	•					•	• •	•			
Diophrys appendiculata	"	O							• • •	• • •	•	• •	• • •	• • •
sp. 4	"	B							•					
sp. 5	?	?	• •	•	• •		•		•		• • •	• • •	• • •	• • •
Abundance	10^3 cells ml^{-1}		0.10 0.21 0.03	2.59 0.81 0.03	0.22 0.45 0.87	0.08 0.03 0.04	0.01 0.08 0.10	0.05 0.01 0.09	0.68 0.73 0.43	0.31 0.34 0.48	0.85 0.69 0.60	2.81 0.76 0.32	3.76 1.23 0.93	1.18 1.06 0.51
	cells cm^{-2}		424	2571	2514	174	328	202	2292	1631	2665	3191	5589	3194
Number of taxa	taxa subsample^{-1}		13 19 3	30 29 3	14 28 23	8 4 4	2 6 10	3 1 14	30 41 32	28 29 30	27 45 36	53 39 34	51 44 51	39 39 36
	taxa sampling occasion^{-1}		27	39	35	10	11	15	52	47	58	66	70	59
	taxa sampling site^{-1}				59						95			

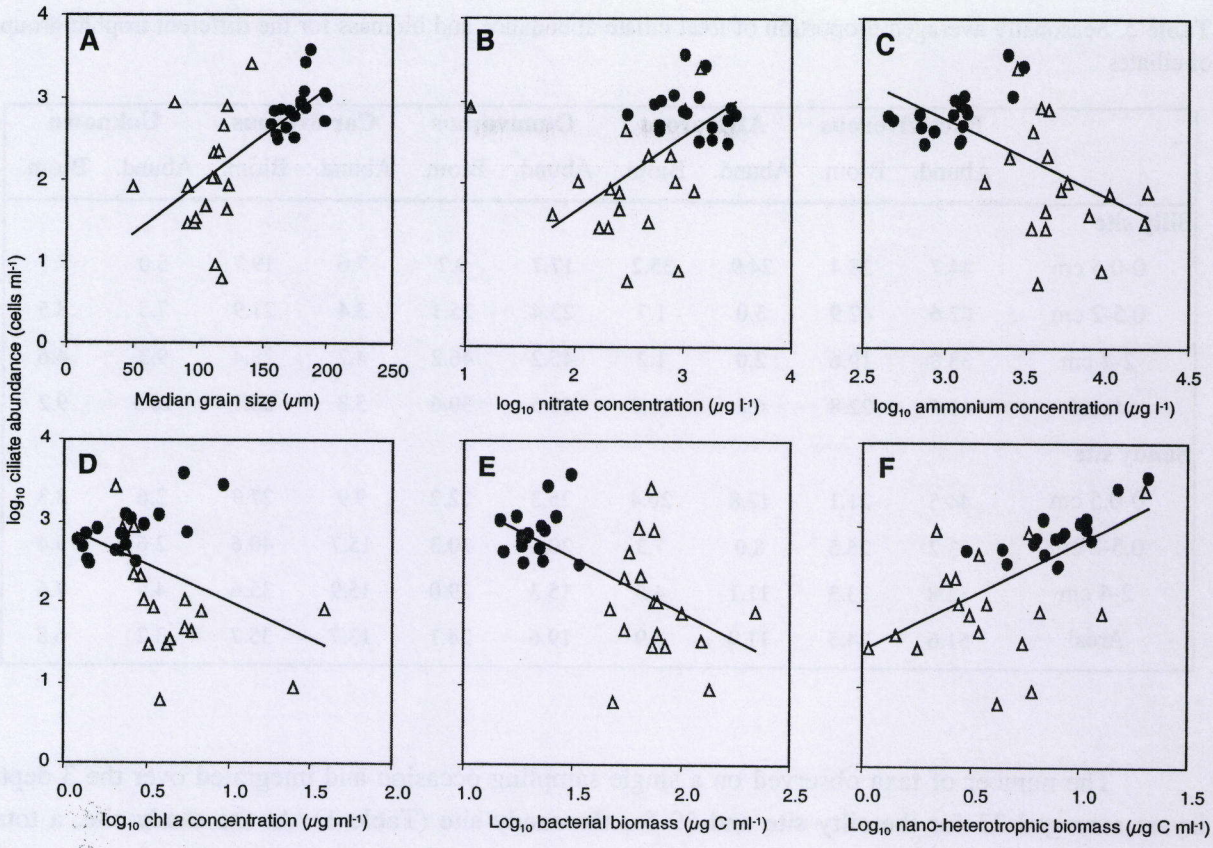

Fig. 3. Relationship between ciliate abundance and (A) median grain size, (B) nitrate concentration, (C) ammonium concentration, (D) chl *a* concentration, (E) bacterial biomass and (F) nano-heterotrophic biomass. Regressions: (A) $y = 0.73 + 0.012x$ ($R^2 = 0.46$, $p < 0.00001$); (B) $y = -0.18 + 0.92x$ ($R^2 = 0.31$ without the deviant sample from the sandy station indicated by a grey symbol filling, $p < 0.001$); (C) $y = 5.62 - 0.94x$ ($R^2 = 0.32$, $p < 0.001$); (D) $y = 2.91 - 0.92x$ ($R^2 = 0.19$, $p < 0.01$); (E) $y = 4.63 - 1.36x$ ($R^2 = 0.40$, $p < 0.001$); (F) $y = 1.46 + 1.32x$ ($R^2 = 0.36$, $p < 0.001$)

The dominant bacterivorous taxa were Scuticociliates and representatives of the genus *Aspidisca* (Table 1). Bacterivores comprised on average about 50 % of areal ciliate abundance at both sites (Table 3). Due to the small size of most bacterivores, their share in ciliate biomass was on average only about 25 % at both sites. Omnivores were proportionally also important, especially at the silty site (Table 3). The relative contribution of algivores was highest at the sediment surface, where they contributed on average to 25 and 18 % of total ciliate abundance at the silty and the sandy site respectively (Table 3). At the sandy site, algivorous ciliates still comprised a large fraction of total ciliate numbers (on average 11 %) in the 2 to 4 cm depth layer, compared to only 2 % at the silty site. Carnivores, mainly Trachelocercids and *Lacrymaria* spp., represented only on average 6 and 14 % of areal ciliate abundance at the silty and the sandy site respectively. Because of their large size, their contribution to ciliate biomass was much higher, on average 26 % at the silty site and 36 % at the sandy site (Table 3). In general, seasonal (not shown) as well as vertical differences in the relative importance of the trophic groups were much higher at the silty compared to the sandy site.

in January (Table 1), and on the ordination diagram are positioned in between the clusters of samples from the 2 sites (Fig. 4A).

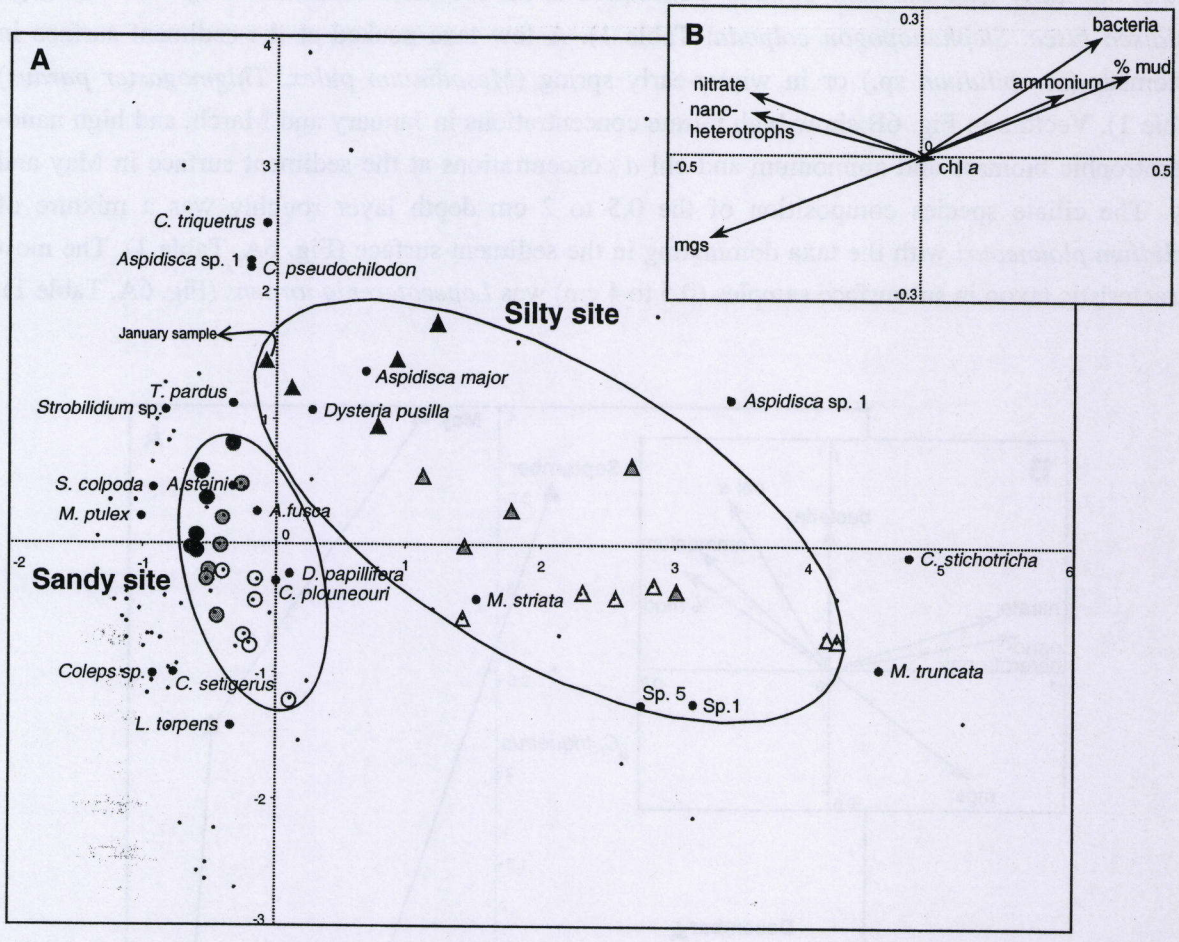

Fig. 4 . (A) CA ordination diagram (first 2 axes) of ciliate communities from the silty and the sandy site, showing the separation of samples in 2 groups defining the 2 sampling sites. Two samples were omitted (see text). Samples from the sandy site are represented by circles, samples from the silty site are represented by triangles. Symbols for samples from the 0-0.5 cm depth layer are black, from the 0.5-2 cm layer are grey and from the 2-4 cm layer are not filled. The ciliate taxa are represented by small black dots. Ciliate taxa accounting for at least 10 % of ciliate abundance in at least 1 sample are named and the dots representing these taxa are larger than the other dots. Full names of the taxa in Table 1. CA axis 1 and 2 together explain 30.8 % of the variation in the species data. (B) Vectors show the direction of increasing values of particular environmental variables

To determine seasonal and vertical differences in the ciliate communities at the 2 sites, samples from both sites were analyzed separately. A CA ordination of samples from the silty site showed that seasonal differences in species composition at this site were much more pronounced at the sediment surface than in the 2 deeper layers (Fig. 5A). Samples from January and May differed most strongly in their species composition. This shift in species composition appears to be related to a high mgs at the sediment surface in January, and a high mud content and high bacterial biomass, and ammonium and chl *a* concentrations in May (Fig. 5B). The taxa listed above as the most characteristic taxa of the silty site were mainly found in subsurface samples, together with *Metacystis striata* and Sp.

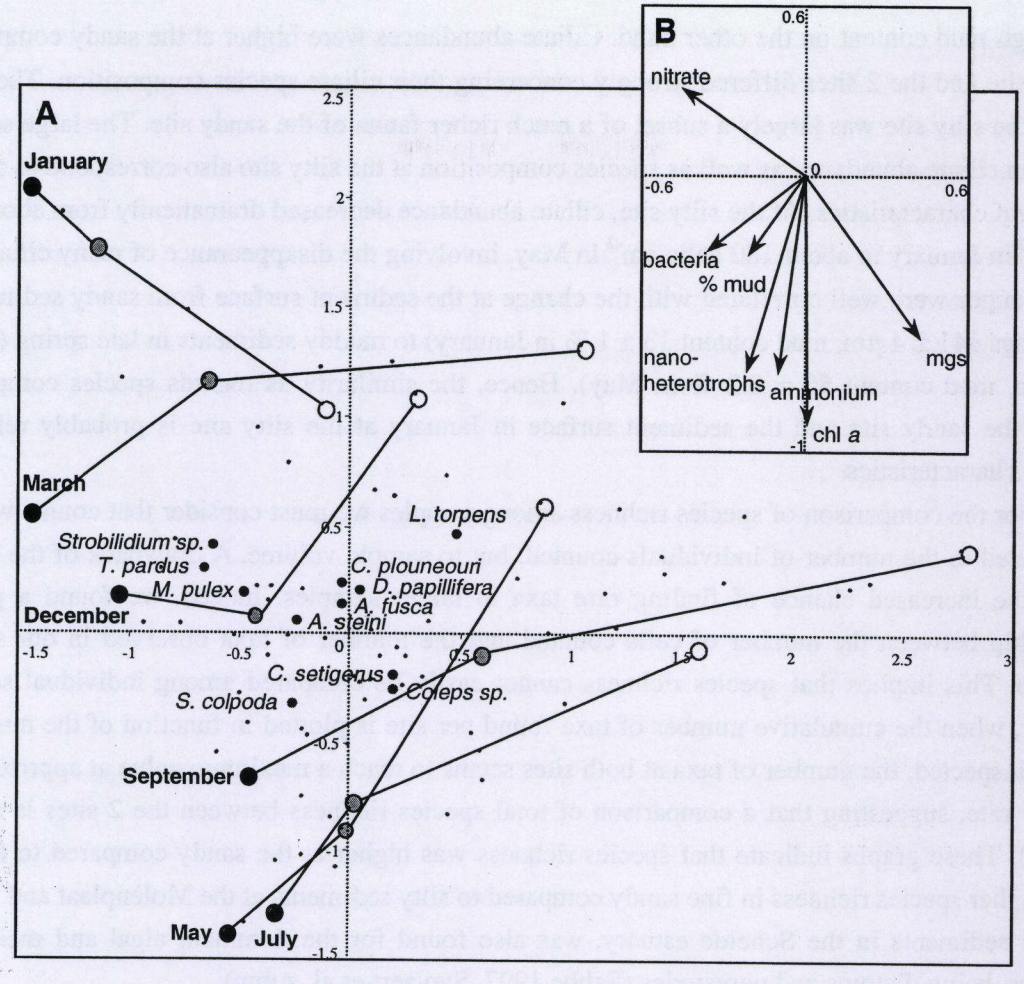

Fig. 6. (A) CA ordination diagram (first 2 axes) of ciliate communities from the sandy site. Symbols as in Fig. 4. Samples from the same sampling occasion are connected by lines and the sampling occasions are indicated. CA axis 1 and 2 together explain 33.3 % of the variation in the species data. (B) as in Fig. 4

Discussion

Ciliate abundances at the Molenplaat intertidal flat ranged from 0.2 to 5.6 x 10^3 cells cm^{-2} integrated over the upper 4 cm of the sediment, and from 0.01 to 3.8 x 10^3 cells ml^{-1} at the sediment surface. These values are within the range of abundances found in marine sediments with similar grain size distributions (Fenchel 1969, Epstein & Gallagher 1992, Epstein 1995, 1997a, b, Al-Rasheid 1997, Wickham et al. 2000, Lee & Patterson 2002). The ciliate community at the Molenplaat also resembled the ciliate fauna typically found in marine sandy sediments. This fauna is either adapted to live in the interstitial space and thus long and worm-shaped like the trachelocercids, or adapted to a dynamic environment and thus small, flattened and rigid, such as *Aspidisca* spp., *Discotricha* sp. and *Thigmogaster* sp. (Fenchel 1969, Patterson et al. 1989).

Sediment characteristics were an important factor regulating the ciliate communities at the Molenplaat. The median grain size (mgs) was the most important predictor of ciliate abundance according to a multiple regression analysis. Moreover, multivariate analyses indicated strong differences in species composition between samples with a high mgs on the one hand and samples

communities (Fenchel 1969, 1987, Alongi 1986, Epstein 1997a). In finer and poorly sorted sediments, clogged interstitial spaces hamper ciliate movement. The fact that large, typical interstitial ciliate taxa such as trachelocercids (Karyorelictida) were almost absent at the silty site at the Molenplaat, illustrates that the amount of interstitial space might have determined part of the differences in species composition among the sites. On the other hand, the robust ciliate species *Metacystis striata* was common in the clogged and compact subsurface sediments of the silty site in spring and summer. Possibly, ciliates in these fine sediments were associated with worm burrows. Ciliates have been observed to use nematode burrows for swimming in sloppy agar which may simulate the density of silty sediment (Jensen 1996).

Sediment characteristics are generally also related to chemical and biological factors such as oxygen-supply and food availability, which have been found to affect ciliate communities in marine sediments (e.g., Epstein et al. 1992, Fenchel & Bernard 1996). Our results suggest that food availability was not a major limiting factor for the ciliates at the Molenplaat. Biomass of bacteria and algae (chl *a*) were significantly negatively correlated to the mgs of the sediment (R = -0.91 and -0.48, respectively) and to ciliate abundances. As a consequence, lower ciliate abundances at the silty compared to the sandy site, and low ciliate abundances at the silty site in summer, could not be explained by a shortage of bacterial or algal food. Possibly, the lower proportion and absolute number of carnivorous ciliates at the silty compared to the sandy site was related to food availability, since the biomass of their main food items, *viz.* ciliates and nano-heterotrophs, was lower at the silty site. Although our results suggest that it was not a major limiting factor for most of the ciliates, food availability was probably involved in seasonal abundance patterns at the sandy site. In contrast to the silty site, physical characteristics of the sediment were not constraining at the sandy site, since the sediment consisted of fine sand throughout the year. Ciliate abundance at the sandy site showed a late spring-summer maximum and a winter minimum. Summer maxima and winter minima are frequently found in marine benthic ciliate communities (e.g., Al-Rasheid 1997, Epstein 1997b, Dietrich & Arndt 2000). This seasonal pattern is the opposite of the seasonal pattern at the silty site and corresponded to a positive correlation with algal biomass (chl *a*) and the biomass of nano-heterotrophs, which are possible food sources of ciliates (Fenchel 1968a). Ciliate abundance at the sandy site was not significantly correlated with bacterial biomass. However, in contrast to bacterial biomass, bacterial production at this site does increase during summer (Hamels et al. 2001b). It is, however, possible that the seasonal changes in the ciliate abundances at the sandy site were merely an effect of temperature on the growth rates of the ciliates (Fenchel 1968b).

In general, fine sediments have a high organic content, a high oxygen demand and a poor oxygen supply (Patterson et al. 1989). Oxygen concentration were not measured in the present study, but higher ammonium and lower nitrate concentrations at the silty compared to the sandy site (Fig. 2) suggest that oxygen concentrations were indeed lower at the silty than at the sandy site. Nevertheless, oxygen concentrations probably had little influence on total ciliate abundances at the Molenplaat. Although ciliate abundances were significantly negatively correlated to ammonium concentrations when all samples were considered together, ciliate abundances were positively correlated with ammonium concentrations at the sandy site. Literature data show that high ciliate abundances have been found in the oxidized zone of marine sands as well as at localities with strongly reducing sediments and a rich growth of sulphur bacteria (Fenchel 1967, 1969). On the other hand, oxygen availability may influence the ciliate species composition since different ciliate species have different

Acknowledgements

This research was performed in the frameworks of the EU Environment & Climate programme ECOFLAT (ENV4-CT96-0216), which is part of the ELOISE programme, the FWO research project no. G.0104.99 and the GOA research project no. 1205398. I. H. acknowledged a grant from the Fund for Scientific Research-Flanders (FWO), K. S. and K. M. are postdoctoral fellows of the same Fund. We thank the Netherlands Institute of Ecology-Centre for Estuarine and Coastal Ecology (NIOO-CEMO) and the crew for the use of the RV 'Luctor'. Profs. W. Foissner and T. Fenchel, and Drs. G. Esteban and K. Al-Rasheid are thanked for their comments on the ciliate identifications. Thanks to Dirk van Gansbeke for the chlorophyll and nutrient analyses. A. Akhiat (National Institute for Coastal and Marine Management/RIKZ, the Netherlands) is acknowledged for the water temperature data.

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Some ciliate photographs

This appendix shows some ciliate taxa from the Molenplaat and aims at illustrating the diversity of the ciliates found on the Molenplaat intertidal flat (Westerschelde estuary, The Netherlands). The ciliates were photographed from the permanent microscopic preparations that were used for the cell counts (see Chapter 3). These preparations are available at the Laboratory of Protistology & Aquatic Ecology, Department of Biology, University of Gent. The ciliates were stained with the silver protein Protargol according to the Quantitative Protargol Stain (QPS) method (see Chapter 3). Protargol reveals the infraciliature (the basal bodies of the cilia and associated structures), the cilia, various fibrillar systems and the nuclear apparatus of the ciliates. Ciliates have a dual nuclear apparatus, with (smaller) micronuclei and (larger) macronuclei (see for instance Plate I, Fig. F and Plate III, Figs. F & H). Individual cilia are sometimes grouped into more rigid structures called cirri, which are mainly used for locomotion (see for instance Plate IV, Fig. F). In addition, ingested diatom frustules are visible in some of the ciliates (see for instance Plate II, Fig. E and Plate III, Fig. G). All scale bars on the pictures are 10 μm in length.

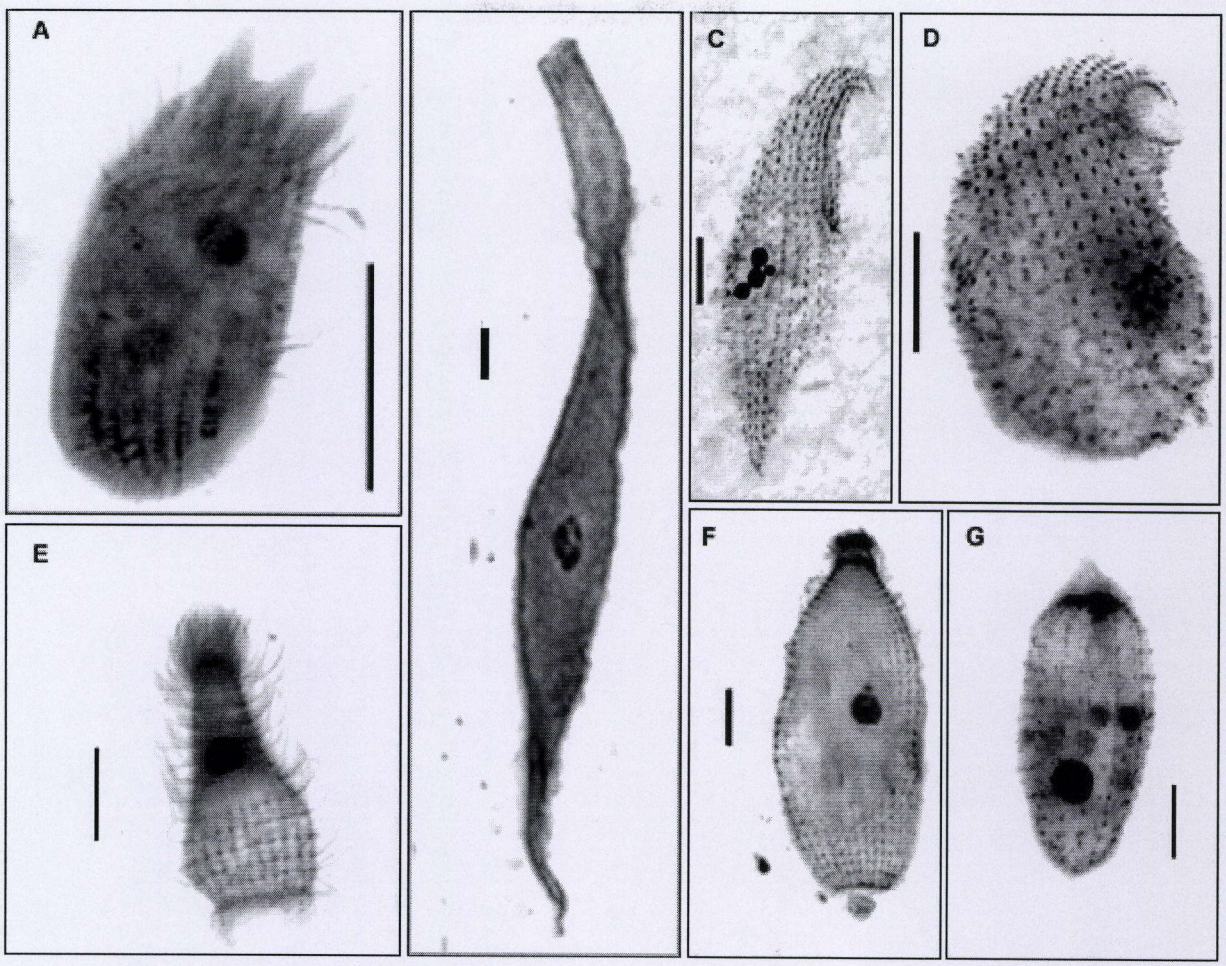

Plate I.

A. *Stephanopogon colpoda* Entz

B. *Tracheloraphis longicollis* (Dragesco) Foissner & Dragesco

C. *Remanella* sp.

D. *Cryptopharynx setigerus* Kahl

E. *Metacystis truncata* Cohn

F. *Metacystis striata* Stokes

G. *Coleps* sp.

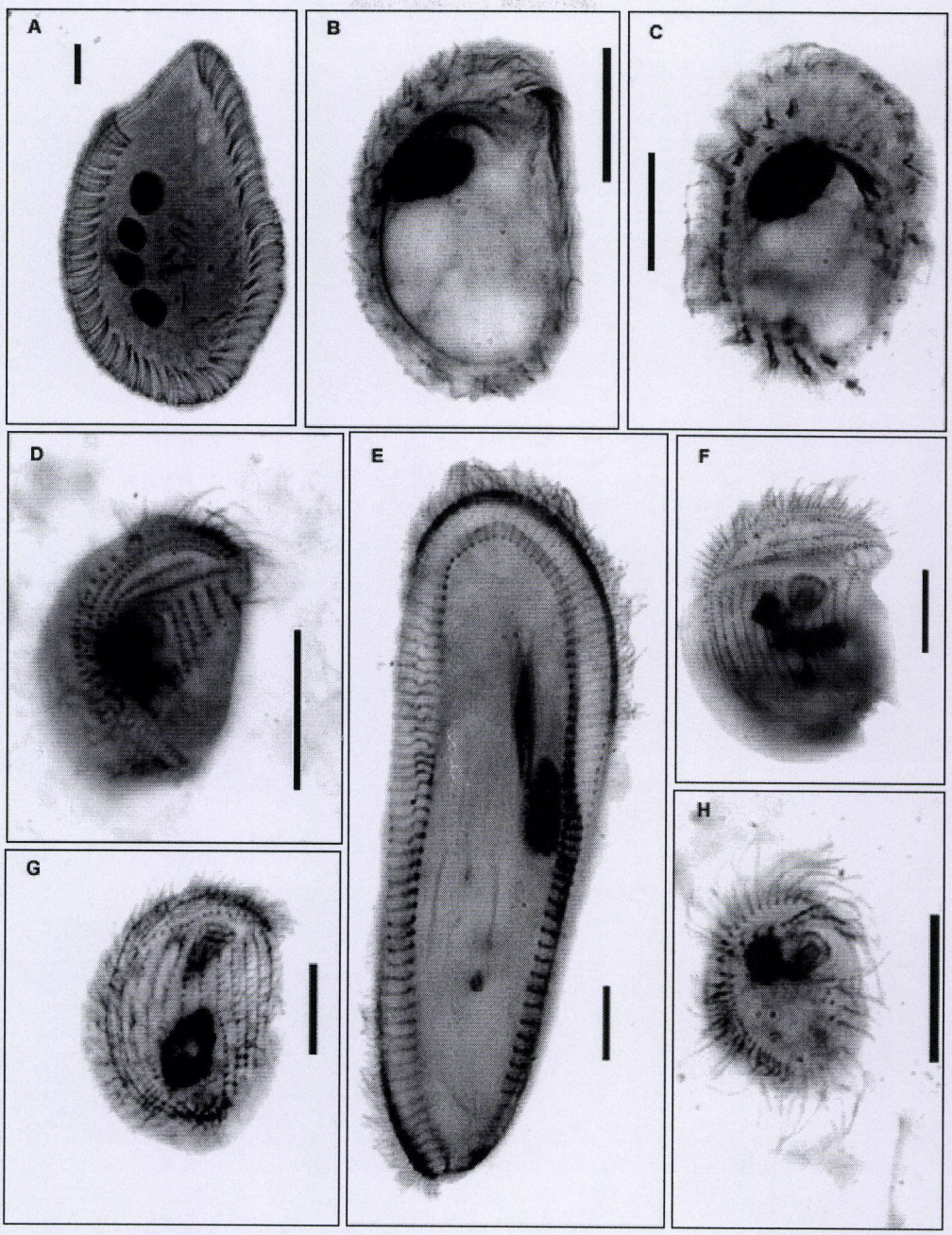

Plate II.

A. *Loxophyllum verrucosum* (Stokes) Dragesco
B. *Lopezoterenia torpens* (Kahl) Foissner
C. *Discotricha papillifera* Tuffrau
D. *Thigmogaster pardus* Deroux
E. *Chlamydodon triquetrus* (Müller) Dragesco
F. *Atopochilodon distichum* Deroux
G. *Chlamydonella pseudochilodon* Deroux
H. *Chlamydonella* sp. 1

Plate III.

A. *Chlamydonella* sp. 2

B. *Lynchella nordica* Jankowsky

C. *Mirodysteria decora* Deroux

D. *Helicostoma notatum* Kahl

E. *Pseudoplatynematum loricatum* Bock

F. *Pleuronema* sp. 1

G. *Hippocomos loricatus* Czapik & Jordan

H. *Cyclidium plouneouri* Dragesco

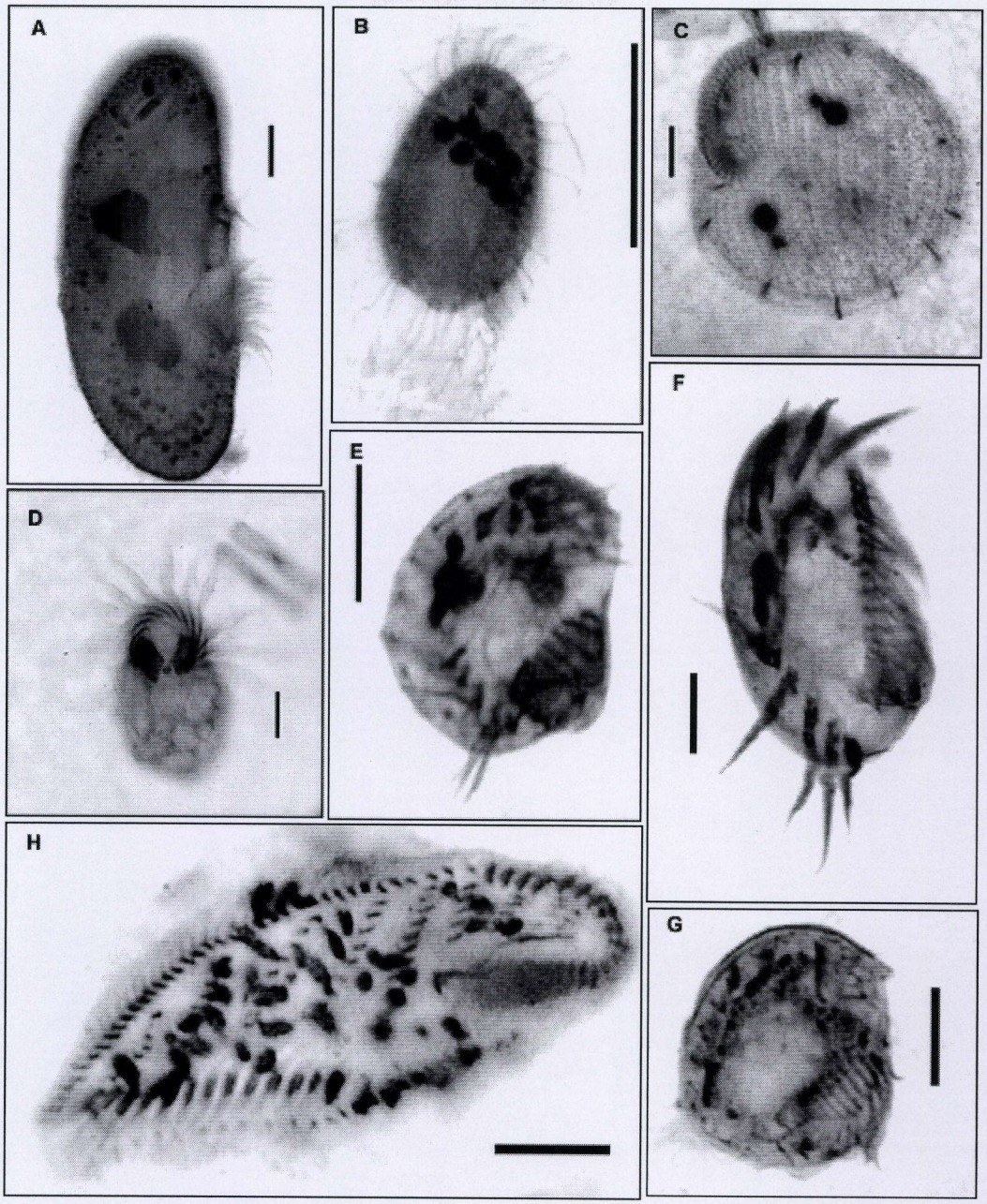

Plate IV.

A. *Histiobalantium marinum* Kahl

B. Sp. 3

C. *Peritromus arenicolus* Dragesco

D. *Strobilidium* sp.

E. *Aspidisca fusca* Kahl

F. *Aspidisca major* (Madsen) Kahl

G. *Aspidisca pulchirrima* Kahl

H. Sp. 4

Chapter 4

Uncoupling of bacterial production and flagellate grazing in aquatic sediments: a case study from an intertidal flat

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Published in
Aquatic Microbial Ecology 25 (1): 31-42 (2001)

Abstract

In contrast to planktonic ecosystems, the fate of bacterial production in aquatic sediments is still largely unclear. In this study, we identified the factors regulating the impact of flagellate grazing on benthic bacterial production for a sandy and a silty intertidal sediment. Flagellate grazing rates were estimated using fluorescently labelled sediment to prevent disturbance of *in situ* bacterial density and community composition and to account for grazing on attached bacteria. Since flagellate cell size was quite diverse, the grazing rates were determined for 4 size classes. Bacterial production was measured simultaneously with grazing estimates. Bacterial density and production increased with decreasing median grain size of the sediment. Bacterial production was strongly related to the chlorophyll *a* content of the sediment, indicating resource control of bacterial production. In contrast to bacteria, flagellate biomass decreased with decreasing median grain size of the sediment. Pairwise comparison of grazing rates between the 2 sites showed that grazing rates were significantly higher at the sandy site. This suggests that the effect of sediment composition on flagellate biomass may be mediated by an influence of sediment characteristics on flagellate ingestion rates. The negative relation of bacterial production and the positive relation of flagellate biomass and grazing rates with median grain size resulted in a significant positive relation between the impact of flagellate grazing on bacterial production and the median grain size of the sediment. These results amount to an uncoupling of flagellate grazing and bacterial production in fine sediments. Our results as well as results from previous studies suggest

In the present study, bacterial grazing by heterotrophic flagellates is compared with bacterial standing stock and production at 2 sites with contrasting sediment characteristics on an intertidal flat in the Schelde estuary. We studied the seasonal variability and identified the factors regulating the impact of flagellate grazing on the benthic bacterial community.

Materials and methods

Study site and sampling

Sediments were obtained from the Molenplaat, an intertidal flat in the polyhaline reaches of the Schelde estuary (SW Netherlands). Two sites (Fig. 1) were chosen on the basis of their sediment characteristics (for full site description see Herman et al. 2000, Middelburg et al. 2000). The silty site (Molenplaat station 2) is located in the central, most protected region of the flat, while the sandy site (Molenplaat station 4) is subject to higher hydrodynamical disturbance.

The experiments were conducted in August, October and November 1998 and March and May 1999. Sediment was collected by hand coring during ebb tide. In the field, the sediment was carefully extruded through the top of the corer and the top 3 mm horizon was sliced off. Samples for bacterial and flagellate densities were taken using 10 ml cut-off disposable syringes (inner diameter 16 mm) with sharpened edges. Sediment slices were pooled and immediately fixed with an equal volume of filtered-sterilized formaldehyde (2.5 % final concentration) (3 slices, bacteria) or ice-cold glutaraldehyde (2 % final concentration) (5 slices, flagellates). For the grazing experiments and the bacterial production measurements, sediment from 25 or 8 plexiglass cores (inner diameter 36 mm) respectively, was pooled and stored at *in situ* temperature. Additional replicate cores of 36 mm were collected for the determination of the grain size distribution (laser diffraction technique using a Coulter® LS 100 with fluid module, Coulter Electronics, Inc.). For the determination of the chl *a* content (according to a slightly modified protocol of Mantoura & Llewellyn 1983), 2 replicate cores of 16 mm were taken and immediately frozen using CO_2 ice.

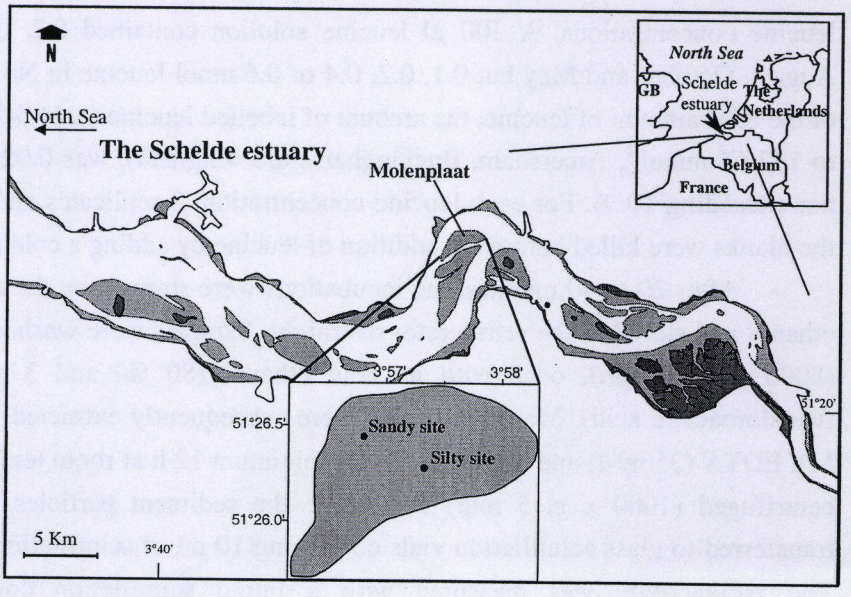

Fig. 1. Schelde estuary, with the location of the sampling sites on the Molenplaat tidal flat

disintegrations per minute (dpm). Quenching was accounted for by automatic external standardization. After subtraction of the blanks, production was calculated using the following equation:

$$\text{Production (mg C ml}^{-1}\text{ h}^{-1}) = dpm \times \frac{60}{t} \times \frac{1}{\left(2.2\times10^{12}\right)} \times M \times \frac{1}{\%Leu} \times \text{C/Prot} \times 10 \times \frac{total\ Leu\ added\ (nmol) + isotope\ dilution\ (nmol)}{SA \times \left[^{3}H\right] Leu\ added\ (nmol)}$$

where t = incubation time (min); 1 Ci = 2.2 x 10^{12} dpm; M = molecular weight of leucine; *%Leu* = fraction of leucine in protein = 0.073; C/Prot = carbon to protein ratio = 0.86 (Simon & Azam 1989); SA = specific activity of the leucine added (Ci $mmol^{-1}$)

Bacterial cell production was estimated using a mean bacterial volume of 0.14 μm^3 per cell (mean value based on Cammen & Walker 1986, van Duyl en Kop 1990, Hondeveld et al. 1992 and Kuwae & Hosokawa 1999) and a conversion factor of 220 fg C μm^{-3} (Bratbak & Dundas 1984).

Preparation of stained sediment

Two days before the actual grazing experiment, sediment was collected at the 2 sites and stained in its entirety (Starink et al. 1994b). Sediment was dispensed in 50 ml centrifuge tubes (Nalgene) and stained with 5-([4,6-dichlorotriazin-2-yl]-amino)-fluorescein (DTAF) during 3 h in a water bath of 60°C. DTAF was dissolved in a 0.05 M Na_2HPO_4/2 % NaCl buffer of pH 9, at a final concentration of 0.2 mg ml^{-1} (Sherr & Sherr 1993b). Unbound dye was removed by repetitive (4 cycles) resuspension/centrifugation (22000 x *g*, 15 min) in the phosphate buffer. After a last rinse with filtered-sterilized Schelde water, the sediment was frozen at –28°C. A few days later it was thawed for the grazing experiment.

Grazing experiments

In acid washed glass containers, freshly collected sediment was gently mixed with stained sediment so that 25 % of the mixed sediment volume was stained sediment. It was placed in a water bath at the temperature of the Schelde water (Fig. 2B) and in the dark. Some filtered (0.22 μm) water was added to facilitate the mixing. As both the stained and the fresh sediment were collected at the same place with only a few days in between, we assume that they have the same bacterial density, which means that 25 % of the bacteria in the mixture was stained. Immediately after mixing, a subsample was taken to check this ratio. For each of the 2 sampling sites, 2 or 3 replicate containers were used.

In a preliminary experiment, the number of ingested FLB per flagellate was found to increase during the first 10 min of the incubation, but not thereafter. Therefore, after 10 min of incubation, a subsample was taken, with a small spoon, from each container. The subsamples were immediately fixed with an equal volume of ice-cold 4 % glutaraldehyde. Flagellates were extracted, stained and collected on filters as described above. They were examined under epifluorescence illumination. UV-excitation was used to locate the flagellates and after switching to blue light excitation, the number of fluorescently labelled bacteria (FLB) inside the cells was counted. Per size class, 50 flagellates were examined. For flagellates larger than 20 μm, all flagellates on a filter were counted if the total number

Table 1. Spearman rank (with Water temp.) and Pearson (other variables) correlation coefficients. mgs: median grain size; mgr: mean grazing rate (see text); %SS: proportion of standing stock grazed d^{-1}; %prod: proportion of daily bacterial production grazed d^{-1}. Levels of significance are * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant

Variable	Water temp	mgs	Chl *a*	Bact dens	Bact prod	Flag dens	Flag biom	mgr	%SS
mgs	ns								
Chl *a*	ns	-0.74*							
Bact dens	ns	-0.96***	0.77**						
Bact prod	ns	-0.72*	0.91***	0.81**					
Flag dens	ns	ns	0.71*	ns	ns				
Flag biom	ns	ns	ns	ns	ns	0.66*			
mgr	ns	0.93***	-0.68*	-0.88***	-0.69*	ns	ns		
%SS	ns	0.85**	ns	-0.86**	ns	ns	0.83**	0.78**	
%prod.	ns	0.82**	-0.67*	-0.88***	-0.82**	ns	ns	0.88***	0.79**

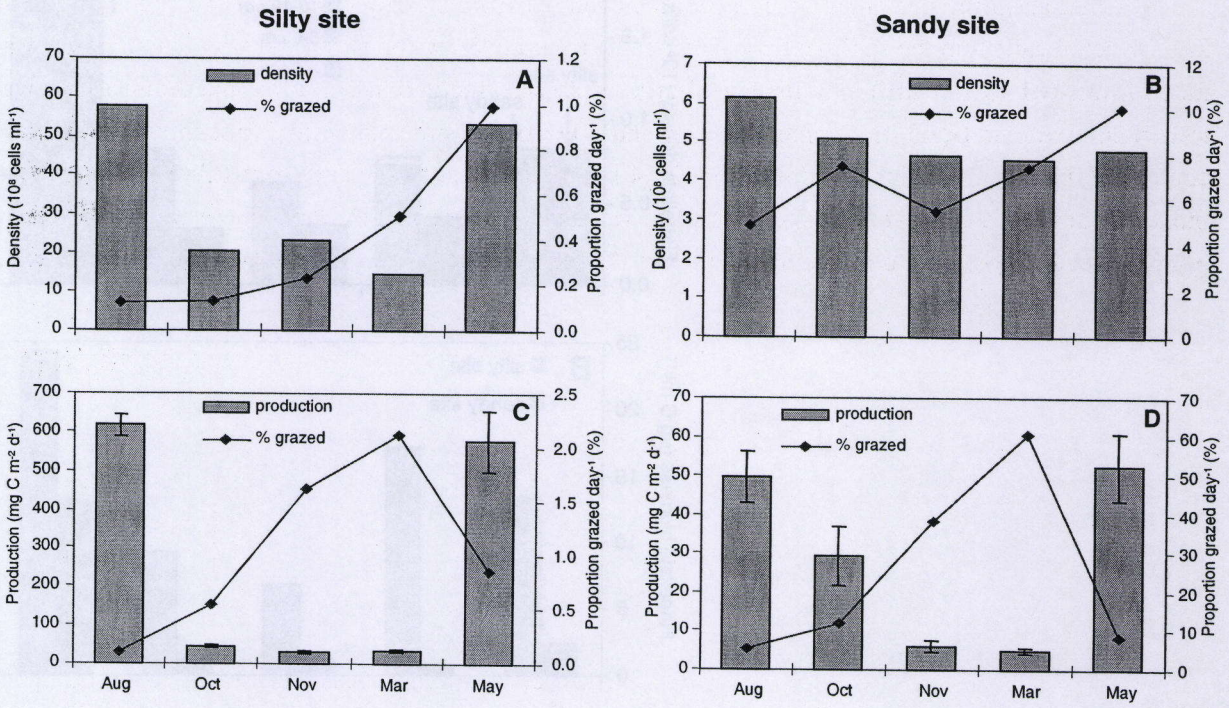

Fig. 3. Bacterial density (A, B) and production (mean ± 1 SD, n = 12) (C, D) at the silty site (A, C) and the sandy site (B, D) on the Molenplaat and grazing by flagellates (% d^{-1})

Bacterial density (Fig. 3A, B) decreased significantly with increasing mgs of the sediment (Table 1). At the silty site bacterial density ranged between 1.5 and 5.8 x 10^9 cells ml^{-1}, with highest numbers in late spring and summer (Fig. 3A). At the sandy site bacterial numbers were always lower, on average 5 x 10^8 cells ml^{-1} or circa 10 % of values at the silty site, with only small seasonal fluctuations (Fig. 3B).

Bacterial production was always significantly higher at the silty site (30 to 618 mg C m^{-2} d^{-1}; Fig. 3C) when compared to the sandy site (5 to 52 mg C m^{-2} d^{-1}; Fig. 3D) (Mann-Whitney *U*-tests, $p < 0.05$). At both sites, seasonal variation was highly significant (Kruskal Wallis, $p << 0.01$), with highest values in May and August and low production in winter and early spring. There was no significant relationship between bacterial production and incubation temperature (Table 1). Bacterial production was significantly positively related to bacterial density and chl *a* content of the sediment, and significantly negatively related to the grain size of the sediment (Table 1). From bacterial densities and production, community growth rate was estimated. At both sites, community growth rate ranged from 0.1 to 1.2 d^{-1}, with values above 1 d^{-1} limited to May and August and lowest values in winter and early spring (not shown).

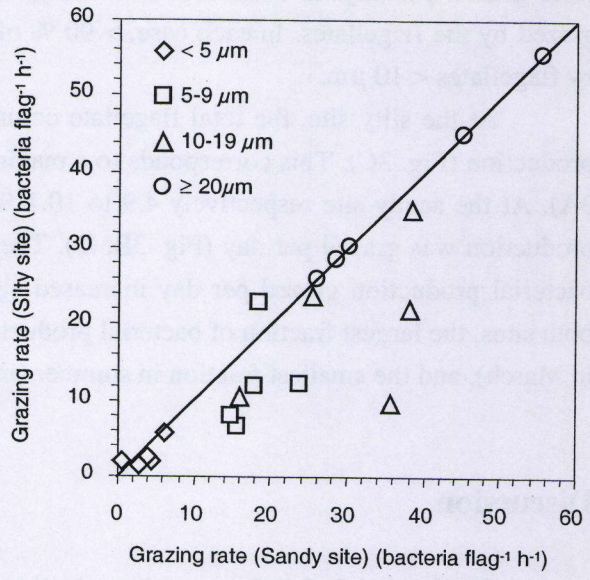

Fig. 5. Comparison of grazing rates at the silty and the sandy site. Grazing rates for flagellates ≥ 20 μm are for the sandy site, presented as if the same grazing rates were found for the silty site. Diagonal line represents the case in which the same grazing rate was found at both sites

Grazing rates were calculated for each flagellate size class separately. At the silty site, the near absence of flagellates > 20 μm made it impossible to calculate the grazing rates for this size class. Based on the relative proportion of the size classes in total flagellate density, the weighted mean of the grazing rates of the different size classes was calculated. For simplicity, the term 'mean grazing rate' will be used.

Grazing rates increased with the size of the flagellates at both sites (Fig. 5). Grazing rates ranged from 0.6 to 6.2 bacteria $flagellate^{-1}$ h^{-1} for flagellates < 5 μm to 26.1 to 55.6 bacteria $flagellate^{-1}$ h^{-1} for flagellates > 20 μm. Pairwise comparison of grazing rates between the silty and the sandy site (Fig. 5), for data from all experiments and for all size classes, showed that grazing rates were significantly higher at the sandy site (paired *t*-test, $p < 0.02$). The same holds for the mean grazing rates, which ranged from 2.7 to 9.1 bacteria $flagellate^{-1}$ h^{-1} at the silty site and from 10.9 to 20.5 bacteria $flagellate^{-1}$ h^{-1} at the sandy site (Fig. 4C). Mean grazing rate was significantly positively correlated with the mgs of the sediment and significant negatively correlated with bacterial density and production (Table 1). The same correlations were found for the different size classes, although they were only significant for flagellates > 5 μm (not shown). Grazing rates of the different size classes of flagellates, as well as mean grazing rates, did not vary significantly among dates (Kruskall Wallis, p >

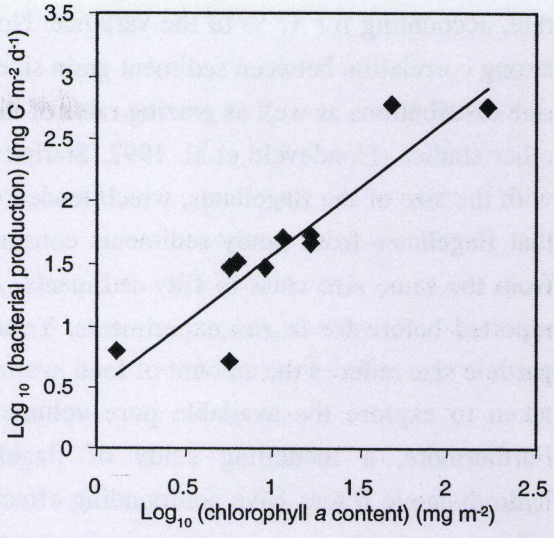

Fig. 6. Relationship between bacterial production and chl *a* content. Regression: $y = 0.49 + 1.09x$ ($R^2 = 0.83$, $p < 0.0003$)

Although flagellate density was in May much higher at the silty site when compared to the sandy site, flagellate biomass was always higher at the sandy site, as larger forms dominated the community at that site. We found no significant relation between flagellate abundance or biomass and bacterial density, bacterial production or temperature. Flagellate biomass was found to be positively related to the median grain size of the sediment. Lower biomass of flagellates in silty compared to sandy sediments was also found in other marine sediments (e.g., Bak et al. 1991, Bak & Nieuwland 1993), although in these cases, the difference did not result from differences in the size distribution of the flagellates, but was entirely attributed to lower densities in silty sediments. Lower flagellate densities in silty sediments were ascribed to small interstitial spaces and adverse chemical conditions, like oxygen stress, prevailing in fine sediments. These circumstances may also explain why larger flagellates were mainly restricted to the sandy site in this study. Information on the effect of sediment characteristics on flagellate distribution is scarce however and very high densities were sometimes found in fine sediments (e.g., Tso & Taghon 1997, this study at the silty site in May). Flagellates in silty sediments may possibly be concentrated temporarily in a small surface layer rich in detritus or carbohydrates, comparable to microbial mats which sometimes harbour high protozoan densities (Bernard & Fenchel 1995). In experiments using artificial sediments, Young et al. (1994) found that the protozoan population decreased as particle size decreased. They suggest that decreasing the particle size reduced the feeding rate and so reduced the rate of population increase. A positive relation between feeding rate and particle size was observed in the present study and will be discussed below.

Our mean grazing rate estimates (2.7 to 20.5 bacteria flagellate^{-1} h^{-1}) are well within the range of values reported for other aquatic sediments (1 to 73 bacteria flagellate^{-1} h^{-1}, in Bot & Kaplan 1990, Novitsky 1990, Epstein & Shiaris 1992b, Hondeveld et al. 1992, 1995, Starink et al. 1994b, 1996a). Highest values were reported by Starink (1994b, 1996a), who attributed this to the use of stained sediment instead of monodispersed FLB and a selection of flagellates for attached bacteria. Using the same method, our grazing rates are several times smaller than the values reported by Starink et al. (1996a) when comparing grazing rates for flagellates of approximately the same size. This suggests that factors other than method and cell size influence grazing rate estimates. In a multiple regression analysis, median grain size was found to be the most important predictor of flagellate mean grazing

Our data, together with literature data suggest that in most aquatic sediments, for a large part of the year, flagellate grazing has no considerable impact on bacterial community dynamics. By contrast, in pelagic communities grazing by heterotrophic flagellates is an important fate of bacterial production (Sherr & Sherr 1994). In planktonic ecosystems both bacterial numbers and flagellate numbers increase with increasing productivity, in space as well as in time, thereby maintaining equilibrium between production of bacteria and flagellate grazing (Berninger et al. 1991, Sanders et al. 1992). In eutrophic waters, predation on flagellates can lead to a temporary uncoupling of bacterial production and flagellate grazing (Berninger et al. 1991, Gasol 1994). Our study suggests that in sediments, bacterial production increases with decreasing median grain size, resulting in high bacterial production in silty and muddy sediments (Fig. 7A). Flagellate biomass, on the other hand decreases with decreasing median grain size of the sediment (Fig. 7B). Increases in bacterial productivity in space as well as over time are therefore usually not accompanied by an equivalent increase in flagellate grazing pressure. As a result, in fine sediments, bacterial production and grazing are strongly uncoupled. In comparison to silty sediments, bacterial production in sandier sediments is lower and flagellate biomass and grazing rates are higher, which results in flagellates having a stronger control over bacterial dynamics (Fig. 7 C, D). A balance between bacterial production and grazing by flagellates is however rarely achieved and is probably restricted to periods when bacterial production is minimal. It seems unlikely that improved estimates of grazing rates for benthic flagellates will change these conclusions.

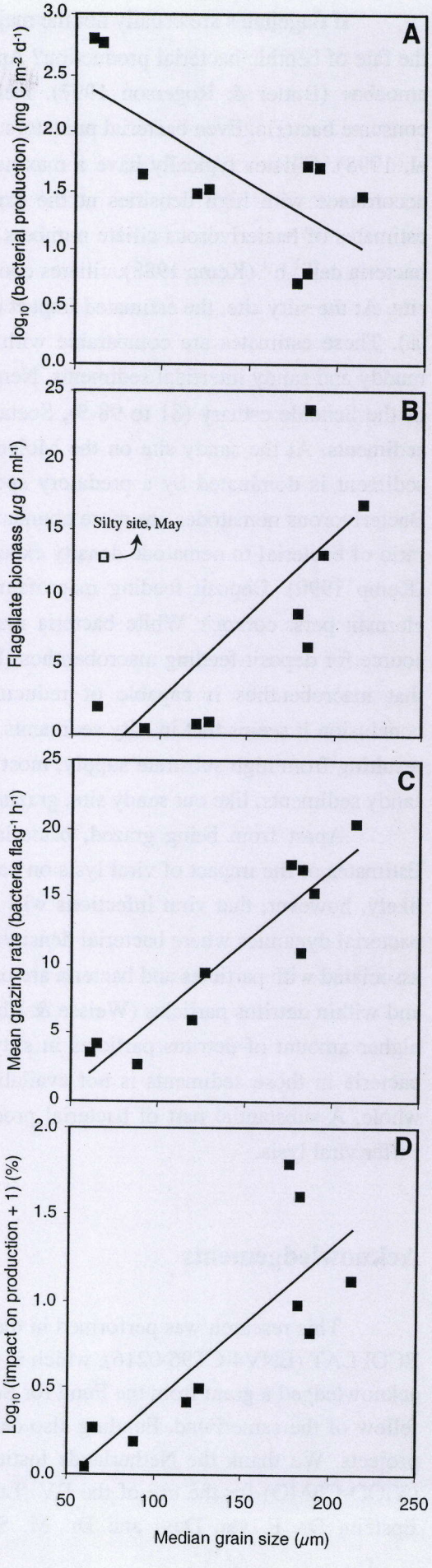

Fig. 7. Relationships between (A) bacterial production, (B) flagellate biomass, (C) mean grazing rate, and (D) the proportion of bacterial production grazed per day by flagellates, and the median grain size of the sediment. Regressions: (A) $y = 2.9 - 0.0089x$ ($R^2 = 0.52$, $p < 0.02$); (B) $y = -9.64 + 0.1215x$ ($R^2 = 0.57$ without the silty site May, $p < 0.02$); (C) $y = -4.74 + 0.11x$ ($R^2 = 0.87$, $p < 0.00008$); (D) $y = -0.46 + 0.0088x$ ($R^2 = 0.67$, $p < 0.004$)

methodology. Thanks to Prof. V. N. de Jonge for valuable comments and to Dirk van Gansbeke for the chlorophyll analyses.

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Chapter 5

Evidence for constant and highly specific active food selection by benthic ciliates in mixed diatoms assemblages

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Submitted manuscript

Abstract

Observational and experimental studies have shown that phagotrophic ciliates, and protozoa in general, are highly selective predators. However, little is as yet known about the actual mechanisms involved in prey selection, and more specifically, about the relative importance of passive selection, governed by the relative availability and vulnerability of the prey items, and active or behavioral selection. We used direct behavioral observations to study the mechanism of prey selection in benthic algivorous ciliates feeding on mixed assemblages of diatom species. Four ciliate species, *viz.* 3 *Strombidium* species and a *Pseudochilodonopsis* species, and 3 diatom species from intertidal sediments in the Schelde estuary were used for the experiments. In each experiment, a single ciliate species was offered a mixture of 2 diatom species. The feeding preferences of the ciliates were estimated, as well as relative encounter rates, attack probabilities and capture successes for the prey species. The feeding preferences were distinctly predator-specific and highly discerning with respect to the nature of the prey species. Passive selection only played a secondary role in the predation of diatoms by the algivorous ciliates studied: the feeding preferences of the ciliates appeared to result mainly from active selection at the encounters stage and, to a lesser degree, also the attack stage of the feeding process. Our observations suggest that selective encounters with the diatoms were caused by non-contact detection of individual prey items, at least for the *Strombidium* species. Additional experiments confirmed that these ciliates were able to distinguish between diatom species on the basis of soluble chemical cues. Grazing was

selection of indivual prey in mixed prey assemblages (Stoecker et al. 1981, Taniguchi & Takeda 1988).

The available data on selective feeding of benthic algivorous ciliates are derived from the analysis of food vacuole contents, which are compared to the composition of the available diatom species (McCormick 1991, Epstein et al. 1992, Finlay et al. 1993, Balczon & Pratt 1995). However, this approach gives only limited insight in the mechanisms involved in individual cell selection and the relative importance of passive and active selection. In the present study, direct behavioral observations were used to study the mechanisms of prey selection in benthic algivorous ciliates feeding on mixed assemblages of diatom species from estuarine intertidal sediments. The feeding preferences of 4 ciliates species were established, as well as relative encounter rates, attack probabilities and capture successes in various 2-species prey mixtures. The influence of prey ratio, prey abundance and feeding history was also determined.

Materials and methods

Organisms for the experiments

The ciliate and diatom species used in our experiments originated from 2 intertidal locations in the polyhaline reaches of the Schelde estuary (SW Netherlands): the Molenplaat intertidal flat and the Paulina salt marsh. The Molenplaat tidal flat was the study site for the ECOFLAT (Eco-metabolism of an estuarine tidal flat) project. A site description of this tidal flat is given in Herman et al. (2001). The Paulina salt marsh is situated downstream of the Molenplaat (Moens et al. 2002).

Three diatom species were used in our experiments (Table 1): *Staurophora salina* (W. Smith) Mereschkowsky, *Navicula phyllepta* Kützing and *Navicula arenaria* Donkin var. *rostellata* Lange-Bertalot. For convenience, the latter will be referred to as *N. arenaria* in the text. The species were isolated from Molenplaat sediments and grown semi-continuously in unialgal, non-axenic batch cultures with f/2-medium (Guillard, Sigma-Aldrich Corp., St. Louis, MO) prepared with filtered (Whatman GF/C) and autoclaved Schelde water. Diatom species were identified and monospecificity of the cultures was verified after oxidation of the diatom valves (Sabbe 1993). Permanent slides of these oxidizes diatoms have been deposited in the permanent slide collection of the laboratory. For each diatom species, the dimensions of 50 cells were measured with an ocular micrometer; biovolume was calculated on the basis of formulas in Hillebrand et al. (1999) (Table 1). An elliptic prism shape was assumed for *Staurophora salina*. For both *Navicula* species, the biovolume was calculated as the mean of the biovolumes based on an elliptic and an parallelogram-based prism. Diatoms were grown in sterilized 1 l glass Erlenmeyer flasks. To provide a solid substratum for the benthic diatoms, the bottom of each flask was covered with a layer of washed and oven sterilized (170°C, 4 h) sand grains (median grain size 168 μm) from the Molenplaat. The flasks were incubated in an incubator with a 12 h light:12 h dark cycle at 10 or 16°C and shaken daily to prevent diatoms clumping together and to remove diatoms from the glass walls and sand grains. New diatom cultures were started frequently (few days to weekly, depending on the species) by subsampling in order to provide exponentially growing diatoms for the ciliate cultures (see below) and for the experiments. For the ciliate cultures, diatom suspensions were poured directly form the culture flasks. For the experiments, diatoms were harvested from the cultures by concentration on a 3 μm Nuclepore Polycarbonate filter. The cells were

growth on suspensions of single diatom species revealed that the 4 ciliate species differed strongly in their ability to grow on a certain diatom species (Table 1). Diatom prey species were considered to be an 'unsuitable' prey species for a given ciliate species if all attempts to sustain a culture of the ciliate species on this diatom species failed. Prey-predator combinations for the experiments were chosen on the basis of these culture experiences. Preceding an experiment, ciliates were (unless stated otherwise) precultured for several generations on a mixture of the 2 diatom species used for the subsequent experiment (see below), even if 1 of these species was unsuitable as a single prey species. These cultures were started a few days before the experiments to yield ciliates in a healty, exponential growth phase.

General experimental conditions

Foraging behavior of the ciliates was observed in Petri dishes of which the bottom (~21 cm^2) was covered by a thin agar layer (Difco agar noble 1.5 %, added to f/2-medium). In each experiment, a mixture of 2 diatom species, at a certain density and biomass ratio, was presented to 1 ciliate species (Table 2). Diatom suspensions from both prey species were combined so that for all experiments (unless stated otherwise), 1 ml of the diatom mixture contained a total diatom biovolume of ~2.9 x 10^8 μm^3 ml^{-1}. One ml of the 2-species mixture was dispensed on the agar slants. Since diatoms settled, this resulted in a diatom biovolume of ~1.4 x 10^7 μm^3 cm^{-2}, corresponding to 4 x 10^3 to 8 x 10^4 cells cm^{-2}. The use of this arbitrary, but fixed amount of diatom biomass, aimed at standardizing among experiments with differently sized diatom species and at minimizing overlap among cells, which would otherwise complicate the observations. The ciliates were starved for 8 to 12 h at 10°C before each experiment to standardize the conditions and to assure prey uptake during observations; they were acclimated to room temperature during 1 h before the experiments.

During preliminary observations, the ciliate species were found to adopt typical raptorial feeding, capturing each particle individually. This feeding mode predominates if the prey:predator size ratio exceeds about 0.1 (Fenchel 1987). The following predation sequence was discerned and defined on the basis of these preliminary observations: *Strombidium* spp. frequently interrupted their helical swimming pattern to stop in the close proximity of a diatom cell (= Encounter or E). Subsequently, the ciliate either swam away without any attempt to ingest the diatom (only E), or started to engulf the diatom (= Attack or A; at least a part of the diatom enters the ciliate cell). Complete ingestion without loss or rejection of the diatom, which was always followed by resumption of swimming, was defined as a Capture (or C). *Pseudochilodonopsis* spp. usually creep or 'walk' along sediment particles. These ciliates walked on the agar surface in a very characteristic pattern resembling circles, and ignored a lot of the diatom cells they 'walked over'. An encounter (E) was defined as an interruption of the normal creeping pattern, lingering some seconds while the diatom was apparently examined. Attack (A) and Capture (C) were defined as for the Strombidium spp.

To start the experiment, a few tens of ciliates (in ~1 ml) were carefully added to a Petri dish with settled diatoms by pipetting. The swimming or creeping ciliates were observed using a dissecting microscope with transillumination. Once an encounter was noticed, this interaction was followed until the ciliate swam away, carefully noting the diatom species involved and whether the encounter was followed by an attack and capture or not. This procedure was repeated during 1 h. The number of encounters registered during a 1 h observation period averaged 99. For each of the 20 treatments (see below and in Table 2), interactions between predator and prey were observed for mostly 4, sometimes 2, consecutive periods (replicates) of 1 h. For each replicate, fresh agar slants, and prey and predator

In a second series of 3 experiments (expts 4, 5 & 6), we examined the feeding behavior of ciliates offered a mixture of 2 diatom species that, on the basis of earlier culture experiences, proved both to be suitable prey for the ciliate species (Table 2). In experiment 4, *Strombidium cinctum* was offered a mixture of *Navicula arenaria* and *Navicula phyllepta* (Table 2). Five different prey ratios, ranging from a density ratio of 1:100 in favor of *N. phyllepta* to 10:1 in favor of *N. arenaria*, were used to test for possible switching behavior. In case of no switching, the preference remains constant as the ratio of the prey available changes. Experiment 5 used the same ciliate and diatom species as experiment 4 and tested the influence of feeding history on selective feeding. Both prey species were offered in biomass-equivalent mixtures (1:1), but ciliates were precultured for several generations with a mixture of both prey species (as for the other experiments) or either *N. phyllepta* or *N. arenaria* as a sole prey species. In experiment 6, the influence of the total amount of diatom biomass on selective feeding behavior was tested with *Strombidium sauerbreyae* offered a mixture of *Staurophora salina* and *N. arenaria* at an equal biomass ratio (Table 2). Total biovolumes were 1.4×10^7 μm^3 cm^{-2} (as for the other experiments), 1.8×10^6 or 1.8×10^8 μm^3 cm^{-2}.

Data analysis

Prey preference was analyzed using Chesson's α selectivity index (Chesson 1983),

$$\alpha = \frac{r_i / p_i}{\sum_i r_i / p_i},$$

where p_i is the proportion of diatom species i in the offered mixture of 2 species, and r_i is the proportion of the captured diatoms that belongs to species i. This index varies between 0 and 1 and is unaffected by the relative abundance of food types, thus allowing meaningful comparisons between treatments with different prey ratios (Lechowicz 1982). The null hypothesis for no preference (i.e., $\alpha_1 = \alpha_2 = 0.5$) was tested by calculating a *t*-statistic (Chesson 1983).

The observed (capture) preferences are the product of the relative encounter rates, attack probabilities (the probability of an attack after an encounter; A/E) and capture successes (the probability of a capture after an attack; C/A) (Sih 1993). The relative number of encounters with both prey species was compared to the number of encounters expected based on the relative abundances of the prey species, using a heterogeneity *G*-test (Sokal & Rohlf 1995). Significance of differences in attack probabilities and capture successes between the prey species was tested using *t*-tests. Between treatment differences in Chesson's index (α), the relative number of encounters, A/E and C/A were tested using 1-way ANOVA. The Student-Newman-Keuls multiple comparisons test was used for *post hoc* pairwise comparisons. These statistical analyses were performed with STATISTICA 5.1 for Windows (StatSoft Inc., Tulsa, OK, USA). Chesson's indices, attack probabilities and capture successes were arcsine square root transformed to meet the normality and variance equality criteria.

Additional experiment

The behavioral observations strongly suggested that the *Strombidium* spp. were able to disciminate between diatom species on the basis of soluble chemical cues (see below). An additional experiment was set up to test this hypothesis. This experiment was performed with the 2 ciliate and diatom species combinations used for experiments 1 and 2 of the behavioral observations (see Table

Results

Mixtures of suitable and unsuitable prey

The observations in the first 3 experiments yielded comparable results. The unsuitable diatom species were not captured at all (Table 3), even when they outnumbered the other prey species with a factor 25, 270 or 130 in terms of biomass, respectively (see Table 2). This means that *Strombidium cinctum* only ingested *Navicula arenaria*, whereas *Strombidium* sp. and *Pseudochilodonopsis* sp. only ingested *Navicula phyllepta*. Consequently, the value of Chesson's selectivity index α for these suitable prey species always equalled 1. The fact that unsuitable prey were not captured did not result from failure of attacks, since the observations revealed that the unsuitable prey were not even attacked (Table 3). Although encounters with both prey species were observed in each experiment, encounters with the unsuitable prey species never resulted in an attack (i.e., A/E = 0). Attack probability after an encounter with a diatom of the suitable prey species, on the other hand, averaged 0.38 ± 0.16 for *Strombidium cinctum* with *Navicula arenaria* as prey, 0.1 ± 0.02 for *Strombidium* sp. with *Navicula phyllepta* as prey and 0.17 ± 0.04 for *Pseudochilononopsis* with *N. phyllepta* as prey (not shown).

The relative number of encounters increased with the relative abundance for both prey species in the 3 experiments (Fig. 1). However, in each case, the suitable prey species was encountered more than expected on the basis of its relative abundance (Fig. 1, Table 3). This difference between the observed and expected encounter proportions was highly significant (*G*-test, all $p < 0.005$), except for 2 of the 3 treatments in the experiment with *Pseudochilodonopsis* sp. (expt 3). The differences were most pronounced when the suitable prey were rare: for the treatment in each experiment with the lowest numbers of suitable prey, the observed relative encounter rates for the suitable prey were a factor 21, 9.4 or 3.6 higher, respectively, than expected on the basis of their relative abundances (Table 3).

Table 3. Total number of encounters, attacks and captures observed in expts 1, 2 & 3 (for the 3 replicates together), and relative numbers of with suitable prey species (average of the replicates)

Expt	Suitable prey offered	Encounters		attacks		captures	
	(% of total number)	total number	% with suitable prey	total number	% with suitable prey	total number	% with suitable prey
1	33.3	257	96.0	48	100	22	100
	3.8	65	47.7	14	100	8	100
	2	62	42.2	12	100	9	100
2	96.4	731	99.5	90	100	90	100
	50	278	98.2	25	100	25	100
	9.1	103	85.1	7	100	7	100
3	92.9	709	96.2	79	100	65	100
	50	631	53.8	55	100	49	100
	9.1	633	33.2	44	100	39	100

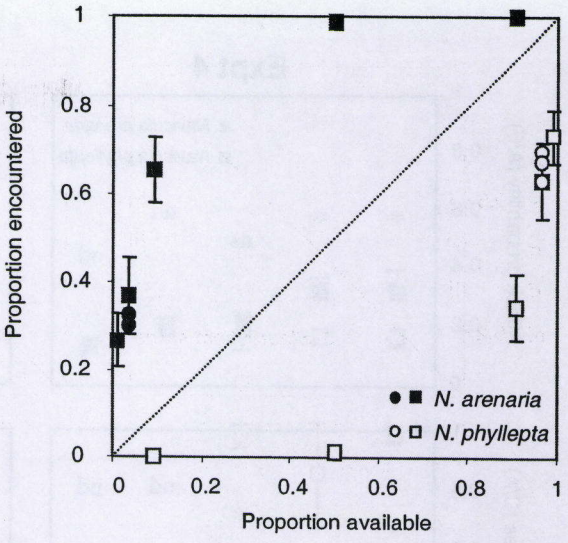

Fig. 3. Proportion of either of the prey species encountered by the ciliates (mean ± 1 SD, n = 4) in relation to its relative abundance (as a proportion) in expts 4 (squares) and 5 (circles). The dotted line represents random encounters. Black boxes represent the suitable prey species; unfilled boxes represent the unsuitable prey species

The relative number of encounters with either of the prey species increased with its relative abundance (Fig. 3), and was not significantly affected by differences in feeding history (Anova, $p > 0.5$). *Navicula arenaria*, the preferred prey species, was encountered significantly more than expected on its relative abundance for all prey ratios and for different feeding histories (*G*-test, all $p < 0.005$; Fig. 3). At equal abundances for both prey species, for instance, only on average 1.2 ± 1.5 % of the observed encounters was with *Navicula phyllepta* (Fig. 3).

Although encounters of *Strombidium cinctum* with *Navicula arenaria* resulted more often in an attack than encounters with *Navicula phyllepta*, the difference was not always significant (Fig. 4). Attack probability averaged 0.27 ± 0.09 with *N. arenaria* and 0.19 ± 0.03 with *N. phyllepta* (averaged for expts 4 and 5), and was not significantly affected by food history (Anova, $p > 0.1$). The attack probability with *N. arenaria* increased when its relative abundance decreased (Fig. 4). Although encountered and attacked less frequently than *N. arenaria*, *N. phyllepta* was practically always captured whenever attacked: capture success ranged from 0.85 to 1 for this prey species (Fig. 4). Capture success for *N. arenaria* averaged 0.35 ± 0.13, was highest when ciliates were precultured with *N. arenaria*, but was not significantly affected by prey ratios or culture conditions (Anova, $p > 0.1$)

In the last set of experiments, *Strombidium sauerbreyae* was offered equal-biomass mixtures of the diatom species *Staurophora salina* and *Navicula arenaria*; total biomass was varied. *S. sauerbreyae* preferentially captured *S. salina* (Fig. 2). Due to high variability between the replicates, the preference for *S. salina* was not significant for the treatment with the lowest total prey biomass (*t*-test of Chesson's index 0.71 ± 0.21 versus 0.5, $df = 3$, $p > 0.1$), but the preference was significant for the 2 other treatments ($p < 0.05$). Nevertheless, Chesson's α did not significantly differ among the treatments (Anova, $p > 0.05$).

Analogous to the previous experiments, the preferred prey species in this experiment was encountered significantly more by its grazer than expected on the basis of its relative abundance (*G*-test, all $p < 0.005$; not shown). Whereas the relative abundance of *Staurophora salina* was fixed at 66.7 % of total abundance in this experiment, the relative number of encounters with *S. salina* averaged 79.6 ± 0.5 % and was not significantly affected by the total amount of prey biomass (Anova, $p > 0.5$).

corresponding to the observation that the preferred prey species were encountered more than expected on the basis of their relative abundances (see Figs. 1 & 3). Since encounters with the preferred prey species resulted more often in an attack than encounters with the unpreferred prey species in all experiments, mean α_A per experiment was always higher than α_E (range: 0.73 to 1; Fig. 5). A higher attack probability for the preferred prey species was, comparatively speaking, very important in the third experiment with *Pseudochilodonposis* sp., but not in the other experiments (Fig. 5). Only in expt 6, the magnitude of the preference for the preferred prey species increased after the attack. Summarizing, the contribution of higher attack probabilities, and especially of higher capture successes to prey preferences (α) was small relative to the importance of preferential encounters (Fig. 5).

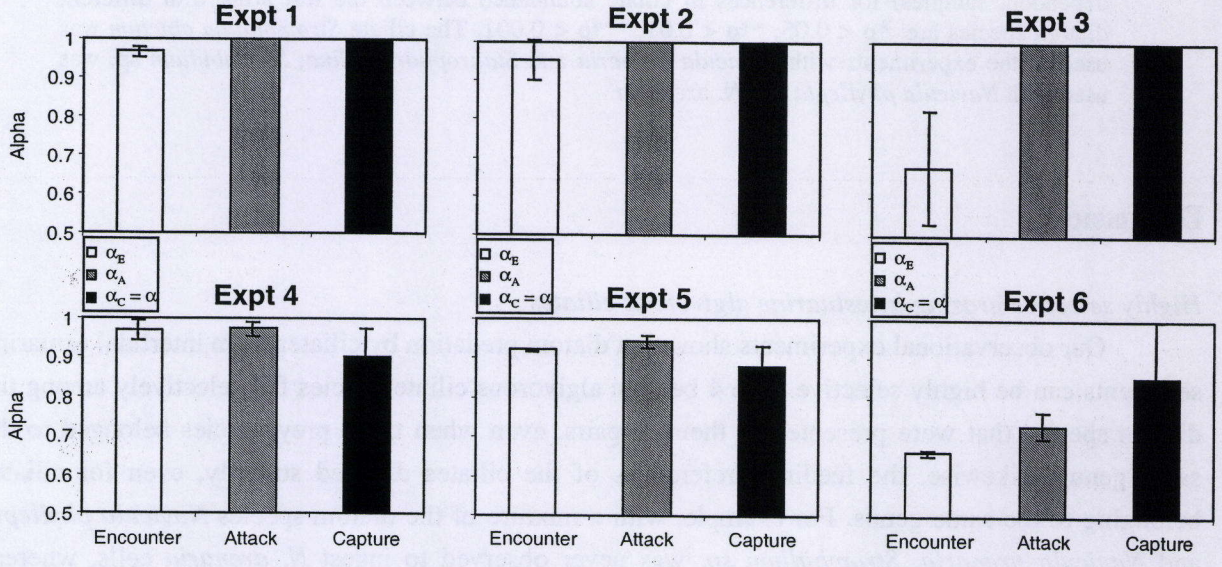

Fig. 5. Preferences evaluated after each step of the feeding process, using derivatives of Chesson's coefficient of selectivity (α = preference after capture), *viz.* α_E (i.e., preference after encounter) and α_A (i.e., preference after attack). Values are means of the average values for each treatment in the experiment ± 1 SD

T-maze experiments

Strombidium cinctum as well as *Strombidium* sp. numbers were significantly higher in the test arms with a suspension of suitable diatoms (*Navicula arenaria* and *Navicula phyllepta*, respectively) than in the test arms with unsuitable diatoms (Fig. 6A). Ciliate numbers were also significantly higher in the test arms with diatom free fluid derived from these suspensions of suitable diatoms (Fig. 6B). When the suspensions were filtered over a 0.2 μm filter, *Strombidium* sp. numbers were still significantly higher in the test arms with *N. phyllepta* compared to *N. arenaria* (*t*-test for dependent samples, $p < 0.05$; not shown).

however, based on direct behavioral observations, allowed us to dissect the actual mechanisms involved in prey selection during the feeding process, and more specifically to evaluate the relative importance of active versus passive selection mechanisms in benthic ciliates.

Importance of active versus passive selection mechanisms in estuarine algivorous ciliates

Finlay et al. (1993) suggested that benthic algivorous ciliates select diatoms merely on a mechanistic basis. McCormick (1991) quoted size differences as well as growth form and microspatial distribution of the diatom species as possible explanations for differences in the consumption of diatom species by ciliates. Growth form and prey microlocation were the same for all diatom species in our experiments. However, also relative prey abundance and prey size did not play a primary role in determining prey selection. Our experiments show that passive selection, governed by the relative availability and the vulnerability of the prey items only played a secondary role in prey selection by the algivorous ciliates. Moreover, the feeding preferences of the ciliates appeared to result mainly from active selection at the *encounter* stage and, to a lesser degree, also the attack stage of the feeding process.

Evaluation of the preferences after encounter, attack and capture of the diatoms revealed that the feeding preferences were mainly established at the encounter stage. The preferred prey species were encountered more than expected on the basis of their relative abundances in all experiments, and this appeared to be the major determinant of the observed feeding preferences for the *Strombidium* species. For *Pseudochilodonopsis* sp. however, preferential encounters with the preferred prey species were only significant when these diatoms were scarce relative to diatoms of the other prey species. Supposing size differences between the diatom species influenced encounter rates, the larger of the 2 diatom species would be expected to have a higher relative encounter rate than expected on the basis of its relative abundance. However, in 3 out of the 5 prey-predator combinations in our experiments (expts 2, 3 & 6), the smallest of both prey species was preferred and encountered more than expected on the basis of its relative abundance. In another experiment (expt 1), the larger *Navicula arenaria* was preferred above the smaller *Staurophora salina*. However, the small size difference between these prey species (approx. a factor 2) cannot explain why the larger *N. arenaria* was encountered on average 12.2 times, and up to 21 times more than expected on the basis of its relative abundance. Accordingly, for the 4 ciliate species in our experiments, neither the relative abundance nor the size of the diatom species explains the preferential encounters with the preferred diatom species. Finally, we can also safely assume that possible differences in the gliding velocities between the diatom species hardly influenced the encounter rates between the ciliates and the diatom species. Diatom movement in general is very slow compared to ciliate speed (by a factor 10^2-10^3; Hay et al. 1993, Fenchel 1987). In conclusion, the observed encounter patterns can only be explained by assuming that the ciliates recognized the diatoms before an encounter and subsequently actively favored encounters with preferred diatoms and/or actively avoided encounters with the unpreferred prey species.

The fact that none of the encounters with an unsuitable prey species resulted in an attack, demonstrates that diatoms were also actively selected after encounter and before an attack, and shows that this selection was well defined and therefore also important. Likewise, in the experiments with 2 suitable prey species, the preferences established at the time of encounters were invariably consolidated by higher attack probabilities for the preferred diatom species (Fig. 5). However, compared to the preferential encounters, a higher attack probability for the preferred diatom species

filtration) derived from diatom cultures unambiguously showed that the *Strombidium* ciliates were able to actively distinguish between different, closely related diatom species without any physical contact between prey and predator. In order to rule out contact between predators and possible prey-associated satellite micro-organisms, which have been shown to be species-specific in marine diatoms (Schäfer et al. 2002), an additional filtration (0.2 μm filtration) was carried out. Even after removing possible associated bacteria, the ciliate *Strombidium* sp. was still able to distinguish between the diatom species. However, at present it remains impossible to assess whether the actual recognition was based on diatom cues or chemical cues exudated by satellite microbes. Ricci et al. (1996) showed that a predatory ciliate was able to distinguish between closely related ciliate prey without any physical contact between prey and predador (using T-mazes), but to our knowledge, this ability was not shown before for algivorous ciliates and closely related algal prey.

The observed chemosensory behavior might be comparable to the behavioral responses involved in several other ecological processes in ciliate communities, such as orientation in oxygen gradients (Fenchel & Bernard 1996), location of prey patches (Fenchel & Jonsson 1988, Fenchel & Blackburn 1999, Morelli et al. 1999) and supposedly also in the congregation of ciliates from complementary mating types (Stock et al. 1999). In all these processes, the ciliates respond to chemical cues by changing their locomotory behavior, ultimately leading to a considerable increase or reduction of motility. The present study highlights the extreme fine-tuning of the chemosensory mechanisms involved in prey recognition, which allows at least some ciliates to detect individual diatom cells in mixed assemblages through non-contact recognition, and not only the accumulation in patches of high prey abundances (as in Fenchel & Jonsson 1988, Morelli et al. 1999).

Conclusion

The present study suggests that trophic interactions between algivorous ciliates and diatoms are far more complex than generally thought. Our experiments unambiguously show that the ciliates were highly selective and distinguished between similar and phylogenetically closely related diatom species in mixed assemblages. The feeding preferences were distinctly predator-specific and prey ratio, total prey density nor feeding history influenced the feeding preferences. Our results show that the feeding preferences resulted mainly from active selection at the encounter stage and, to a lesser degree, also the attack stage of the feeding process. Combined results from observations and T-maze experiments suggest that species-specific soluble chemical cues were involved in the selection of individual prey cells, at least for the *Strombidium* species.

The pronounced specificity of diatom predation by the ciliates in the present study, and the recognition by the ciliates of chemical cues excreted by the diatoms, should be kept in mind for the design of grazing experiments. For instance, grazing rate estimates obtained by the addition of only a single diatom species as prey (see for instance in Epstein 1997) should be treated with caution. Likewise, experimental consumption rates for dead versus living (i.e., excreting) diatoms by ciliates might differ. Apart from possible toxicity of the dyes, heat killing during staining in Balczon & Pratt (1995) might have accounted for the preferential ingestion by ciliates of unstained diatoms, offered together with stained diatoms.

Although the available data are scarce, it is generally assumed that protozoa do not have an important quantitative impact on diatom standing stocks in benthic marine and estuarine ecosystems (Epstein et al. 1992, Hamels et al. 1998, Lee & Patterson 2002). Some studies, on the other hand, have

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Chapter 6

Trophic interactions between ciliates and nematodes from an intertidal flat

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Published in
Aquatic Microbial Ecology 26 (1): 61-72 (2001)

Abstract

The present study investigated the possibility of a trophic link between ciliates and nematodes in fine sandy sediments of the Molenplaat intertidal flat (Schelde estuary, SW Netherlands). Grazing experiments were conducted under controlled laboratory conditions, with ciliate species isolated from enrichment cultures and nematodes collected directly from the field. Significant reductions in ciliate numbers were found in the presence of the predatory nematode *Enoploides longispiculosus*, a prominent species (and genus) in fine to medium sandy sediments of the North Sea and adjacent estuaries. No such effects were found when ciliates were inoculated with a mix of mainly deposit-feeding nematodes from the same sampling site. On the basis of these results, ciliate predation by *E. longispiculosus* was tested for several benthic ciliate species and abundances, at a range of predator abundances and temperatures, and in the presence of alternative prey (*in casu* nematodes). *E. longispiculosus* significantly reduced densities of 5 out of 6 ciliate species offered as prey. Depending on the experimental conditions and the prey species, predation rates ranged from 0.19 to 10.8 ciliates predator^{-1} h^{-1}, corresponding to a biomass consumption of 0.001 to 0.33 μg C predator^{-1} d^{-1}. An overall positive relation between available ciliate biomass and predation rate was found. Comparison of experimental data with field conditions suggests that a considerable part of the ciliate production in fine sandy sediments of the Molenplaat is likely to be consumed by *E. longispiculosus*, which largely dominates meiofaunal biomass there. Estimated carbon requirements for the predator and production estimates of ciliate and

Wickham et al. 2000). In the Schelde estuary (SW Netherlands), ciliate abundances ranging from 1.6 to 5.6 x 10^3 cm^{-2}, corresponding to a biomass of 2.4 to 12.6 μg C cm^{-2}, were found in fine sandy sediments of an intertidal flat (Hamels et al. 1998, Hamels pers. obs.). In nearly all estuarine and marine sediments, nematodes are numerically the dominant metazoans. This also holds true for intertidal sediments of the Schelde estuary, where they constitute on average over 90 % of total meiofaunal densities (Soetaert et al. 1994). Although both ciliates and nematodes are numerous in marine fine sandy sediments, and although some nematode species were shown to be capable of ingesting ciliates (von Thun 1968, Bouwman et al. 1984, Moens & Vincx 1997, E. Olafsson pers. comm.), quantitative data concerning nematode predation on ciliates are lacking.

The present study addressed the following question: Can nematode predation on ciliates constitute a significant trophic link in fine sandy estuarine tidal flat sediments? That is, are nematodes capable of regulating ciliate biomass in intertidal sediments and may ciliates contribute significantly to the nematode diet? Feeding experiments were performed under controlled laboratory conditions, with ciliate cultures and nematodes originating from the Molenplaat, an intertidal flat in the Schelde estuary.

Materials and methods

Prey and predatory organisms

The organisms used in our experiments originated from the Molenplaat, an intertidal flat in the polyhaline reaches of the Schelde estuary (SW Netherlands). This tidal flat has been intensively studied in terms of ecological, biogeochemical and physical processes in the framework of the ECOFLAT (Eco-metabolism of an estuarine tidal flat) project. Major emphasis was on 2 study sites with contrasting sediment characteristics (see Middelburg et al. 2000 and Herman et al. 2000 for a description of these sites, S2 and S4). For our experiments, sediment was collected at a fine sandy site (S4 in the aforementioned studies) where ciliates are very abundant (Hamels et al. 1998). Ciliate species were cultured in the laboratory, whereas nematodes were collected from the field before each experiment.

Six ciliate species were used in our experiments (Table 1): the hypotrichs *Aspidisca* sp., *Euplotes bisulcatus* Kahl and *Euplotes mutabilis* Tuffrau, 2 scuticociliates from the genus *Cyclidium* (sp. 1 and 2) and *Chlamydodon triquetrus* (Müller) Dragesco from the order Cyrtophorida. Ciliates were isolated from Molenplaat sediments (after enrichment by the addition of rice grains or diatoms to sediment suspensions; Caron 1993) and grown monospecifically (unless stated otherwise) in Petri dishes containing filtered (Whatman GF/C) habitat water, adjusted to a salinity of 18 with Milli-Q water. Not all these ciliate species are very common on the Molenplaat; rather, they are the most easily cultivated species. *C. triquetrus* is a strictly herbivorous species (Fenchel 1968), which can be temporarily very abundant on the Molenplaat (up to ~500 cells ml^{-1}). The other species were cultured with bacteria as a food source, even though the *Euplotes* spp. are also capable of feeding on small diatom species (authors' pers. obs.). In contrast to *Euplotes*, the genera *Cyclidium* and *Aspidisca* are very common on the Molenplaat (up to ~1100 and 700 cells ml^{-1}, respectively). *Cyclidium* species swim freely in the interstitial water, while the other species are dorsoventrally flattened and usually creep along the surface of sediment particles. Stock cultures were stored at 10°C under a 12 h light:12

species identification was based on Platt and Warwick (1983). Several tens of individuals were observed at high magnification on the occasion of the first sampling; a few more were carefully checked at each subsequent sampling. All but 1 belonged to the species *E. longispiculosus*, in line with its known distribution and abundance in the Schelde estuary and at the present sampling site (Soetaert et al 1994, Steyaert et al 2001). For all our experiments, we selected "large" individuals, i.e., fourth-stage juveniles and adults. Average measures are given in Table 1; they were not determined separately for each experiment. For extraction of nematodes from the sediment, sediment was resuspended in habitat water and vigorously mixed, and the supernatant was decanted over a 63 μm mesh sieve. Nematodes were hand-picked on the tip of a needle , transferred to filtered habitat water and stored overnight at 4°C before the experiments. This procedure strongly reduced the risk of cotransferring ciliates with the nematodes and allowed the transfer all predators to a single experimental unit within 10 min. At the same time starvation was minimal.

Prey and predator abundances used in our experiments are within the range of abundances of ciliates and nematodes in the field.

General experimental conditions and statistical evaluation

Sediment (median grain size 168 μm) from the sampling site was used as a substratum in our experiments. It was washed with running tap water over a 53 μm sieve and oven sterilized at 170°C for 4 h. One gram aliquots of dry sediment were transferred to 2 ml screw-capped test tubes and rehydrated by the addition of 600 μl of ciliate suspension. This resulted in approximately 1 ml of wet sediment. The tubes were then acclimated in an incubator to the experimental temperature (16°C unless stated otherwise) for at least 2 h. Predatory nematodes (30 per experimental unit, unless stated otherwise) were manually transferred to the tubes at time 0. Control tubes received no nematodes. Grazing and control tubes were incubated horizontally for 24 h in the dark. Incubations were stopped by the addition of 1 ml of ice cold glutaraldehyde to a final concentration of 2 %. For each prey species or abundance (see Table 2), 3 or 4 extra replicate tubes were preserved at time 0 to determine exact initial ciliate densities. Just before cell counts, Rose Bengal was added to stain the ciliates. The samples were homogenized and a 1 ml subsample was withdrawn just after settling of the sand particles. Ciliates were counted under a light microscope at 100 x magnification in at least 350 μl of this supernatant using a Sedgwick-Rafter cell. In each experiment, we used 3 or 4 replicate grazing tubes for each treatment (see below and in Table 2) and 3 or 4 control incubations. All samples were analyzed within 1 week after termination of the experiment.

Predation rates were calculated according to Frost (1972), using the following equations:

$$I = \frac{g \times C}{P} \qquad (1)$$

$$g = \ln\left(\frac{C2}{C2*}\right) \text{x } t^{-1} \qquad (2)$$

$$C = \frac{C2* - C1}{\ln C2* - \ln C1} \qquad (3)$$

where I is predation rate (ciliates predator^{-1} h^{-1}), g is grazing coefficient (h^{-1}), t is incubation period (= 24 h), C is mean ciliate abundance during the incubation (assuming exponential increase or decrease of abundances during the incubation period), P is predator abundance (ind. ml^{-1}), $C1$ is initial ciliate

In the first experiment we found no grazing of the mix of (mainly deposit-feeding) nematodes on ciliates. Therefore, all subsequent experiments used *Enoploides longispiculosus* as the predator. In a second experiment, the susceptibility of 5 ciliate species (*Cyclidium* sp. 1, *Cyclidium* sp. 2, *Euplotes mutabilis, Chlamydodon triquetrus* and *Aspidisca* sp.) to predation by *E. longispiculosus* was tested (Table 2). Two different densities of *Cyclidium* sp. 1, *Cyclidium* sp. 2 and *E. mutabilis* were used. The remaining 2 species were reared in a mixed culture yielding only low abundances. Hence, they were offered to *E. longispiculosus* as a 2-species mix at a single density.

The results of the second experiment suggested that predation rates depend on prey density. This effect was examined in more detail in a third experiment with *Cyclidium* sp. 1 as the prey species (Table 2). Initial ciliate abundances were 387 ± 125, 908 ± 168, 2426 ± 355 and 5739 ± 232 cells ml^{-1}. Ingestion rates were related to mean ciliate abundances within the incubation period, as estimated according to Eq. (3).

The main question in the fourth experiment was whether and how predation rates of *Enoploides longispiculosus* on ciliates would be affected by the presence of alternative prey, *in casu* nematodes. This is of particular relevance to the field situation, where alternative prey is always available. *Monhystera* sp., a bacterivorous nematode species heavily preyed upon by *E. longispiculosus* (Moens et al. 2000), was used as nematode prey. These were handpicked from monospecific cultures, originating from a saltmarsh in the polyhaline reach of the Schelde estuary close to the Molenplaat (see Moens & Vincx 1998 for details on culture conditions). *Euplotes mutabilis* was used as the prey ciliate. Initial ciliate abundance was constant (2330 ± 132 ml^{-1}), whereas 5 different prey nematode densities were used: 0, 10, 30, 60 and 100 nematodes per grazing tube (Table 2). Controls contained ciliate and nematode prey at the same densities as in the grazing tubes but did not contain the predator, *E. longispiculosus*. At the end of the incubation, ciliate numbers in control tubes without and with *Monhystera* sp. at different densities did not differ significantly (ANOVA, $p = 0.16$), suggesting no interaction between the prey organisms. Predation rates of *E. longispiculosus* on *Monhystera* sp. were also determined in this experiment. Part of the prey nematodes was counted from the subsamples withdrawn for ciliate counts. The remainder was extracted by decantation after sample homogenization in 1 ml of filtered habitat water. This procedure was repeated 6 times and nematodes were counted in the pooled supernatant using a dissecting microscope. Carbon ingestion was estimated using an individual carbon content of 0.047 μg C for *Monhystera* sp. (Moens et al. 2000, our Table 1). Prey preference was analyzed as described above. Chesson's α selectivity index is unaffected by the relative abundance of food types, thus allowing meaningful comparisons between treatments (Lechowicz 1982).

A final set of experiments aimed at extending the relevance of the observed predation rates on ciliates to field conditions, where, among other factors, predator abundance and temperature are not constant. The separate effects of predator density and temperature on predation rates of *Enoploides longispiculosus* on *Cyclidium* sp. 1 and *Euplotes mutabilis* were tested (Table 2). Four predator densities were used: 10, 30, 45 or 60 per grazing tube. Grazing and control tubes were incubated at 16°C. Additional replicate grazing tubes with 30 predator nematodes and associated control tubes were incubated at 10 and 22°C. Predation rates at 10 and 22°C were then compared to rates obtained at 16°C with a predator density of 30 ind. ml^{-1}.

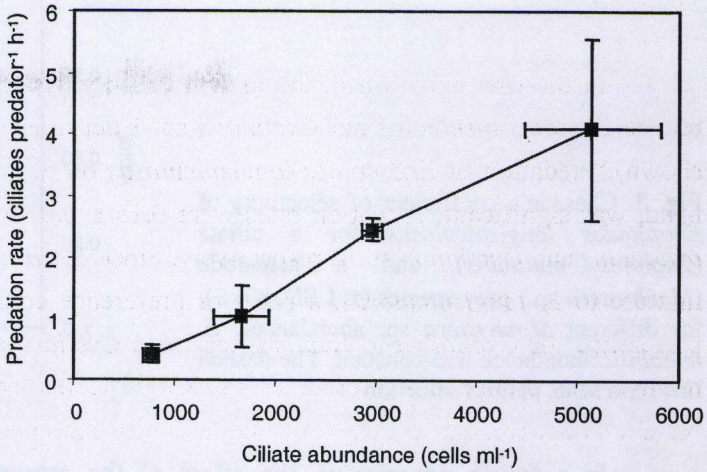

Fig. 1. Predation rates of *Enoploides longispiculosus* as a function of mean abundance of *Cyclidium* sp. 1 over the experimental period. Mean ± 1 SD (n = 3)

In the third experiment, a positive relation between predation rates of *Enoploides longispiculosus* and the density of its prey (*Cyclidium* sp. 1) was found (Fig. 1), but there was no saturation of the predation rate over the range of prey densities tested. Predation rates increased from 0.41 ± 0.14 to 4.1 ± 1.5 ciliates predator^{-1} h^{-1} over a range of 759 ± 79 to 5138 ± 677 ciliates ml^{-1}. Corresponding carbon uptake rates were 0.003 to 0.027 µg C predator^{-1} d^{-1}.

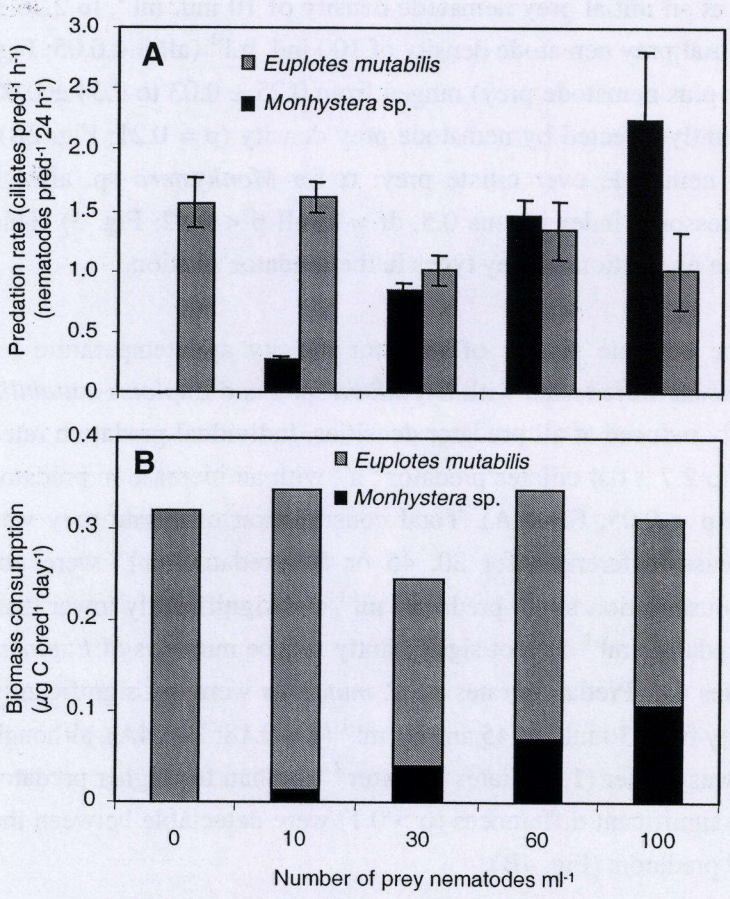

Fig. 2. (A) Predation rates of *Enoploides longispiculosus* on ciliate (*Euplotes mutabilis*) and nematode (*Monhystera* sp.) prey (mean ± 1 SD, n = 3), and (B) total daily biomass consumption (ciliate + nematode prey) for different prey nematode abundances. Initial ciliate abundances were constant (2330 ± 132 ml^{-1}). Pred: predator

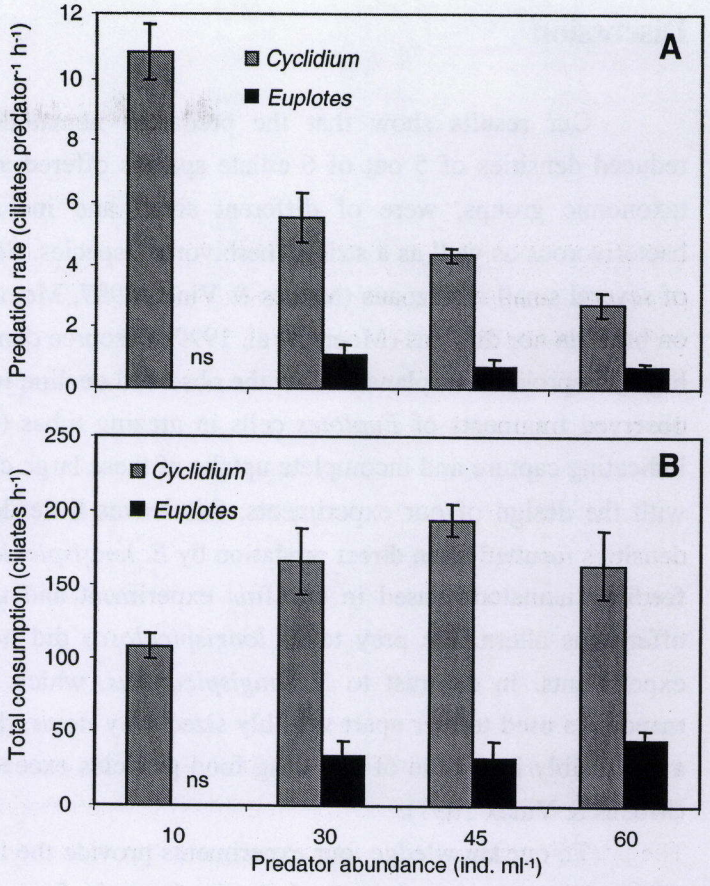

Fig. 4. (A) Predation rates of *Enoploides longispiculosus* on *Cyclidium* sp. 1 and *Euplotes mutabilis* (mean ± 1 SD, n = 3), and (B) total consumption of all predators for both prey species at different *E. longispiculosus* abundances. ns: ciliate numbers in control and grazing tubes were not significantly different

The effect of temperature on predation rates was most pronounced with *Cyclidium* sp. 1 as a prey species. Densities of this species were not significantly reduced by *Enoploides longispiculosus* at 10°C, but they were at 16 and 22°C. At 22°C, the mean predation rate was 25 % higher than at 16°C, but this difference was not significant ($p = 0.26$; Fig. 5). *Euplotes mutabilis* numbers were significantly reduced at all temperatures tested, but temperature differences did not result in significantly different predation rates ($p = 0.35$; Fig. 5). On average, predation rates on *E. mutabilis* were slightly higher at 16 than at 10 and 22°C.

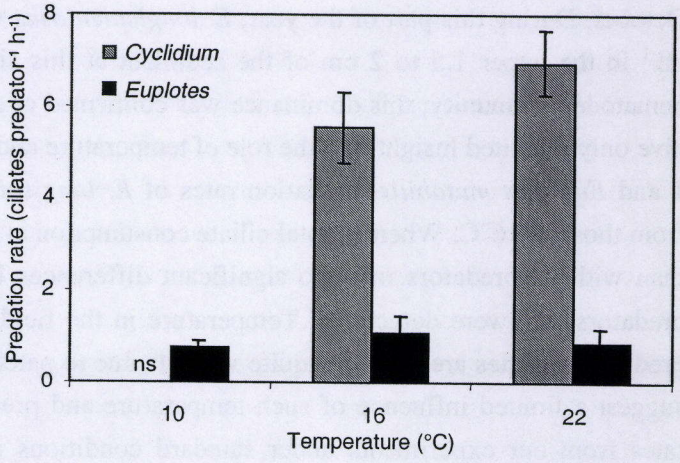

Fig. 5. Predation rates of *Enoploides longispiculosus* on *Cyclidium* sp. 1 and *Euplotes mutabilis* (mean ± 1 SD, n = 3) at different temperatures. ns: ciliate numbers in control and grazing tubes were not significantly different

In our functional response experiment, predation rates of *Enoploides longispiculosus* increased with increasing ciliate (*Cyclidium* sp. 1) density and hence biomass, but there was no saturation within the range of prey abundances used. Although the highest *Cyclidium* sp. 1 density used in this experiment was high compared to field densities of ciliates, ciliate biomasses, viz. 0.1 to 1.6 μg C ml^{-1}, were lower than biomasses found at our sampling site (2 to 3.5 μg C ml^{-1} in the upper 2 cm in late spring-autumn) due to the relatively small size of *Cyclidium* sp. 1. The range of ciliate biomasses used in all our experiments conducted under 'standard' conditions, including those with the large *Euplotes mutabilis* as prey, encompassed a broader range of ciliate biomasses, up to biomasses exceeding those encountered at the Molenplaat. These data show an increase in predation rates up to a biomass of 20 μg C ml^{-1} (Fig. 6), without a clear sign of staturation up to a ciliate biomass of at least 4 μg C ml^{-1}. On the basis of these results and field biomasses for ciliates (see above), a predation rate of about 0.04 μg C predator^{-1} d^{-1} can be expected in the field (Fig. 6). Twenty predators ml^{-1} would then consume about 0.8 μg ciliate carbon ml^{-1} d^{-1}. The impact of this predation on the ciliate community can only be assessed after comparison with ciliate production rates. Maximum growth rates (μ_{max}) for ciliates can be estimated using a multiple regression equation based on extensive laboratory data (Müller & Geller 1993), which relates temperature and species size to growth rate. Maximum production of a mixed assemblage of ciliates can then be estimated by addition of the products of growth rate with biomass for each species. For the ciliate community of the upper 2 cm at our Molenplaat station, in late spring to early autumn, a maximum production of about 2 to 4.5 μg C ml^{-1} d^{-1} is obtained in this way. Given the above-mentioned estimated consumption of ciliate carbon in the field, nematode grazing would amount to about 18 to 40 % of daily ciliate production. This is a conservative estimate, since growth rates in the field are probably lower than the estimated rates (e.g., because of food limitation). In pelagic communities, for instance, *in situ* ciliate growth rates were found to be 2 to 5 times lower than the estimated maxima (Taylor & Johannsson 1991, Leakey et al. 1994, Macek et al. 1996). Nematode grazing may thus be an important, if not the major, fate of ciliate production in intertidal sediments at our sampling site on the Molenplaat.

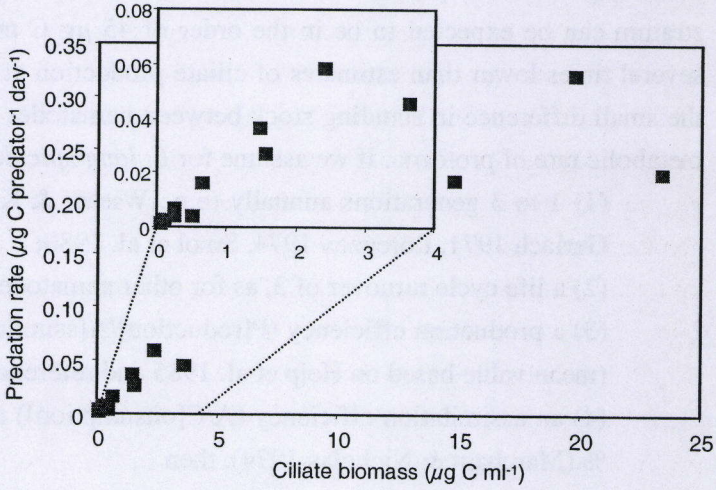

Fig. 6. Predation rate as a function of ciliate biomass for all experiments conducted under standard experimental conditions

(0.001 to 0.33 μg C predator^{-1} d^{-1}). This comes down to a carbon flux of 0.33 to 9.9 μg C ml^{-1} d^{-1} for the *E. longispiculosus* population at our sampling site at the extant summer density. Both upper and lower values are extremes (as are the experimentally determined ingestion rates), with a value of about 1.5 μg C ml^{-1} d^{-1} probably being a plausible average (assuming 2 generations annually and A/C = 40 %). Even if we consider the prey nematode production/biomass of 9 as a conservative estimate (Vranken et al. 1986), this strongly suggests that prey nematode populations at the study site on the Molenplaat provide insufficient carbon to sustain the extant predator population. Hence, at least in summer, ciliates are probably a far more important carbon source for *E. longispiculosus* than nematodes. This implies that carbon transfer from primary producers and bacteria to predatory nematodes may be mediated largely by the microbial food web. Comparison of these values with ciliate production also suggest that, even in the presence of alternative prey, a considerable part of ciliate production is probably consumed by *E. longispiculosus* in the field.

The fate of predatory nematode carbon in Schelde sediments is at present not very clear. Li et al. (1996) modeled temporal fluctuations in nematode (whole community) densities at different sites in the estuary and concluded that they are regulated primarily by macrobenthic infauna. So far, there are no experimental studies supporting this claim. On the other hand, surface-dwelling meiofauna has long been shown to be important prey to epi- and hyperbenthic fish and crustaceans (see, among others, Gee 1989, Coull 1990). Harpacticoid copepods are generally considered to be more susceptible to predation by sediment-dwelling fauna, but this view may be partly biased because nematode remains are not easily recognizable in gut content analyses. The large (3.3 to 4.2 mm long, 76 to 145 μm wide) predacious *Mesacanthion diplechma*, a nematode showing clear preference for superficial sediment layers, was disproportionately abundant in sand goby guts in sediments of the Southern Bight of the North Sea and of the nearby Oosterschelde Estuary (Hamerlynck & Vanreusel 1993). Whether such trophic relations also exist in the Schelde estuary and, if so, how important they are in terms of carbon fluxes from the microbial food web to higher trophic levels remains to be established.

Our experiments, as well as the situation at the sampling site on the Molenplaat, are, of course, a case study, the general importance of which remains to be established. However, densities of ciliates at our study site are generally comparable with those found in other marine and estuarine fine sandy sediments (Fenchel 1967, Al-Rasheid & Sleigh 1995, Epstein 1997, Wickham et al. 2000). *Enoploides longispiculosus* and other members of this genus occur in high densities (comparable with those found on the Molenplaat) in this type of sediments in the North Sea (Skoolmun & Gerlach 1971, Vincx et al. 1990), the Schelde estuary and some other European estuaries (Platt & Warwick 1983, Li & Vincx 1993, Soetaert et al. 1994, 1995). Since we know of no other reports of a similar to that found in the upper 2 cm of the fine sandy sediment on the Molenplaat, the (nematode) prey limitation of *E. longispiculosus* at this site may be unusual. However, Warwick (1971) found that nematodes in muddy sediments in the Exe estuary tended to be small and mainly deposit feeders, while species from sandy sediments tended to be predators or epigrowth feeders with long bodies. Hence, density and relative abundance of supposedly predatory nematodes are probably typically much higher in sandy than in silty sediments. Hence, in view of the generally high densities and biomasses of ciliates as well as of predatory nematodes in sandy sediments, nematode predation on ciliates probably constitutes a significant trophic link in many estuarine and marine sediments.

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Summary

Estuarine ecosystems are of high ecological value because they are very productive, harbour a great diversity of organisms and act as a buffering filter for land derived wastes on their way to the coastal zone (Heip et al. 1995, Abril et al. 2002). Intertidal sediments play a crucial role in the carbon cycle of meso- and macrotidal estuaries and are an important site for accumulation and mineralization of organic matter in these estuaries. High amounts of allochtonous organic matter and high *in situ* primary production in intertidal sediments are the basis of a complex food web with micro-, meio-, and macrobenthic consumers. Although the role of meiobenthic, and especially the macrobenthic animals received considerable attention, the quantitative importance and role of microbenthic consumers, i.e., the ciliated, flagellated and amoeboid protozoa in intertidal estuarine sediments, and aquatic sediments in general, is as yet largely unknown. Nevertheless, due to their high functional diversity and high potential growth rates, protozoa have the potential to fulfil an important ecological role in aquatic ecosystems. In pelagic ecosystems, protozoa have been recognized as major consumers of bacteria and as an important food source for many zooplankton species (Stoecker & Capuzzo 1990, Sanders et al. 1992). Methodological problems connected with the extraction of protozoa from sediments and masking by sediment particles, however, caused a backlog in the knowledge of benthic compared to pelagic protozoa. In spite of methodological improvements in the last 15 years, the available quantitative data on benthic protozoa remain as yet limited, not only in terms of their spatial and temporal coverage, but also in terms of their habitat. This can partly be ascribed to the complexity of extraction and enumeration methods for benthic protozoa, and the complexity of benthic ecosystems. Likewise, the study of trophic interactions in the benthic microbial food web is more complicated than in pelagic environments because organisms live in close proximity of each other and in close association with particles. As a consequence, data on the role of protozoa as grazers of benthic carbon sources, and as a food source for higher trophic levels in sediments are very limited at present.

The aim of the present study is to contribute to the knowledge of the role of protozoa in intertidal estuarine sediments. The research in this study focussed on protozoa from intertidal sediments in the Schelde estuary. A prerequisite to estimate the potential role of protozoa in these sediments is knowledge of their quantitative importance. Since this knowledge is very limited at present, a first aim of the present study was to provide data on the quantitative importance and the composition of protozoan communities in intertidal Schelde sediments, their spatial and temporal distribution patterns and the factors potentially regulating these dynamics (chapters 2 & 3). In addition, some aspects concerning the potential role of benthic protozoa as consumers of the available benthic carbon resources (chapters 4 & 5) and as a food source for higher trophic levels (chapter 6) were studied experimentally.

Quantitative importance and composition of protozoan communities, spatio-temporal patterns and potentially regulating factors

A 1-year study investigated the quantitative importance of protozoa in intertidal sediments of a polyhaline (the Molenplaat intertidal flat) and a freshwater (mud flat at Appels) site in the Schelde

whereas the ciliate fauna in subsurface sediments was poor but changed little throughout the year. Species richness and abundance of the ciliate community were higher at the sandy station. Moreover, seasonal and vertical dynamics were less pronounced at this station. Ciliate abundances at the sandy station changed gradually from a winter minimum to a maximum in summer. Simultaneously, the vertical distribution pattern of the ciliates shifted upwards. The spatio-temporal distribution patterns at the Molenplaat suggest that sediment characteristics were an important factor regulating the ciliate communities at this site. The differences between the sandy and the silty station at the Molenplaat, and the seasonal patterns at the silty station demonstrate that physical properties of the sediment were more important for the ciliates than food availability or temperature. The results from the sandy Molenplaat station suggest that temperature and the availability of food and oxygen were probably involved in the regulation of seasonal and vertical dynamics of the ciliates when physical characteristics of the sediment were not constraining.

The role of protozoa

The importance of protozoa relative to other benthic consumers

The relative importance of protozoan and meio- and macrobenthic consumers in the carbon and energy flow in sediments is hardly studied. A first idea about the importance of protozoa is given by their biomass. The biomass of the protozoa obtained during this study, was compared to biomass data for metazoa for the period late spring (Molenplaat; P. M. J. Herman, M. Steyaert pers. comm.) or early autumn (Appels; J. Seys pers. comm). Protozoan biomass exceeded the biomass of metazoa at the sandy Appels station, but was low (< 5 %) compared to the combined biomass of meio- and macrobenthos at the other sampling stations. Nevertheless, due to a higher turnover of smaller cells, the contribution of protozoa to benthic energetics is disproportional to their biomass. Protozoan and metazoan biomasses at the 4 sampling stations in late spring/early autumn and estimates of their weight-specific metabolic rates suggest that protozoa accounted for ~29 to 96 % of the combined metabolic rate of benthic consumers at that time. The estimated relative metabolic rate of the protozoa was higher at the sandy than at the silty stations at Appels and the Molenplaat, and was mainly accounted for by the nano-heterotrophs. These rough estimates suggest that protozoa should be recognized as a full member of benthic ecosystems and emphasize the importance of small protozoa in the sediments studied.

The impact of protozoa on the communities of their prey

In contrast to planktonic ecosystems, the fate of bacterial production in aquatic sediments is still largely unclear. Grazing studied with benthic flagellates are scarce, and mostly reveal only a small impact of flagellate grazing on benthic bacterial production (e.g., Epstein & Shiaris 1992). Nevertheless, it has been suggested that grazing rate estimates have probably been underestimated because grazing on attached bacteria was neglected (Starink et al. 1994). Biomass ratios of bacteria and algae to protozoa suggest that protozoa have a higher grazing impact at the Molenplaat compared to Appels, and at the sandy compared to the silty station at both sites. The impact of flagellate bacterivory at the sandy and the silty Molenplaat station was studied in more detail. Flagellate grazing

The experiments unambiguously show that the ciliates were highly selective and distinguished between similar and phylogenetically closely related diatom species in mixed assemblages. The feeding preferences were also distinctly predator-specific, and neither total prey density nor feeding history influenced the preferences. Grazing was also constant, i.e., prey switching was not observed. The observations show that passive selection, governed by the relative availability and vulnerability of the diatom species only played a secondary role in the predation of diatoms by the ciliates studied. The feeding preferences of the ciliates appeared to result mainly from active selection at the encounter stage and, to a lesser degree, also at the attack stage of the feeding process. The observations also suggest that selective encounters with the diatoms were caused by non-contact detection of individual prey items, at least for the *Strombidium* species. Additional T-maze experiments confirmed the ability of these ciliates to distinguish between diatom species on the basis of soluble chemical cues. The combined results from observations and T-maze experiments suggest that species-specific soluble chemical cues were involved in the selection of individual prey cells, at least for the *Strombidium* species. The highly specialized trophic interactions between ciliates and their diatom prey, as shown by the experiments, may be an important driving force in shaping structure and diversity of benthic diatom communities in intertidal sediments. Moreover, the pronounced specificity of diatom predation by the ciliates in the present study, and the recognition by the ciliates of chemical cues excreted by the diatoms, should be kept in mind for the design of grazing experiments.

Protozoa as a food source for higher trophic levels

Nematodes, which are the most abundant metazoans in most intertidal estuarine sediments, have repeatedly been observed to ingest ciliates. Nevertheless, quantitative data on ciliate predation by nematodes is lacking. The possibility of a trophic link between ciliates and nematodes in fine sandy sediments of the Molenplaat intertidal flat was studied using grazing experiments. These experiments were conducted under controlled laboratory conditions, with ciliate species isolated from enrichment cultures and nematodes (the predatory nematode *Enoploides longispiculosus* or a mix of mainly deposit feeding nematodes) collected directly from the field. Significant reductions in ciliate numbers were found in the presence of *E. longispiculosus*, which is also a prominent species (and genus) in other fine to medium sandy sediments of the North Sea and adjacent estuaries. No such effects were found when ciliates were inoculated with a mix of mainly deposit-feeding nematodes from the same sampling site. On the basis of these results, ciliate predation by *E. longispiculosus* was tested for several benthic ciliate species and abundances, at a range of predator abundances and temperatures, and in the presence of alternative prey (*in casu* nematodes). *E. longispiculosus* significantly reduced densities of 5 out of 6 ciliate species offered as prey. Depending on the experimental conditions and the prey species, predation rates ranged from 0.19 to 10.8 ciliates predator^{-1} h^{-1}, corresponding to a biomass consumption of 0.001 to 0.33 μg C predator^{-1} d^{-1}. An overall positive relation between available ciliate biomass and predation rate was found. Comparison of experimental data with field conditions suggests that a considerable part of the ciliate production in fine sandy sediments of the Molenplaat is likely to be consumed by *E. longispiculosus*, which largely dominates meiofaunal biomass there. Estimated carbon requirements for the predator and production estimates of ciliate and nematode prey at the study site, strongly suggest that ciliates are probably a far more important carbon source for *E. longispiculosus* than nematode prey. This implies that carbon transfer from primary

del Giorgio et al. 1996) needs further investigation. The experiments on the selection of diatoms by ciliates in the present study showed that this interaction is very complex.

The present work provides information on a only limited number of aspects concerning the role of protozoa in intertidal estuarine sediments. A lot of other interesting aspects could not be dealt with because of time limitations, but also because of methodological problems. Further efforts are needed to overcome these methodological problems.

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Samenvatting

Estuariene ecosystemen zijn ecologisch zeer waardevol omdat ze zeer productief zijn, een grote verscheidenheid aan organismen huisvesten en een filter zijn voor afvalstoffen vanop het land voordat deze terechtkomen in de kustzone (Heip et al. 1995, Abril et al. 2002). Intertidale sedimenten spelen een cruciale rol in de koolstofcyclus van meso- en macrotidale estuaria en zijn een belangrijke plaats voor accumulatie en mineralisatie van organisch materiaal. Grote hoeveelheden allochtoon organisch materiaal en hoge *in situ* primaire productie in intertidale sedimenten vormen de basis van een complex voedselweb met micro-, meio- en macrobenthische consumenten. De rol van het macrobenthos, en in minder mate ook het meiobenthos, in intertidale estuariene sedimenten kreeg reeds heel wat aandacht. Het kwantitatief belang en de rol van microbenthische consumenten, dit zijn de gecilieerde, de geflagelleerde en de amoeboide protozoa, in deze sedimenten en aquatische sedimenten in het algemeen is echter nog grotendeels onbekend. Nochtans suggereren hun hoge functionele diversiteit en hoge potentiële groeisnelheden dat protozoa een belangrijke ecologische rol kunnen spelen in aquatische ecosystemen. In pelagische ecosystemen bleken protozoa belangrijke consumenten te zijn van bacteriën en een belangrijke voedselbron voor vele zooplankton soorten (Stoecker & Capuzzo 1990, Sanders et al. 1992). Doordat de aanwezigheid van sediment het bestuderen van benthische protozoa sterk bemoeilijkt kwam het onderzoek naar protozoa minder snel op gang in sedimenten dan in het plankton. Hoewel er voor deze problemen methodologische oplossingen werden gezocht gedurende de laatste 15 jaar, blijft de beschikbare kwantitatieve informatie over benthische protozoa beperkt, zowel wat betreft hun ruimtelijke en temporele dynamiek als wat betreft de bestudeerde habitats. De complexiteit van de methoden voor extractie en voor het tellen van benthische protozoa en de complexiteit van de benthische ecosystemen zelf kunnen dit deels verklaren. Ook het bestuderen van trofische interacties in het microbieel voedselweb is moeilijker in benthische dan in pelagische ecosystemen omdat de organismen veel dichter bij elkaar leven en sterk geassocieerd zijn met de sediment partikels. Daardoor zijn gegevens over de rol van protozoa als consumenten van de benthische koolstofbronnen en als voedselbron voor hogere trofische niveaus momenteel zeer beperkt.

Het doel van deze studie is bij te dragen tot de kennis over de rol van protozoa in intertidale estuariene sedimenten. Het onderzoek in deze studie concentreerde zich op protozoa van intertidale sedimenten in het Schelde-estuarium. Om de mogelijke rol van protozoa in deze sedimenten in te kunnen schatten zijn er in de eerste plaats kwantitatieve gegevens nodig. Omdat deze gegevens vrijwel ontbreken was het eerste doel van deze studie het verzamelen van gegevens over het kwantitatief belang en de samenstelling van de gemeenschappen van protozoa in intertidale Schelde-sedimenten, hun ruimtelijke en temporale verspreidingspatronen en de factoren die deze patronen mogelijk reguleren (hoofdstukken 2 & 3). Bovendien werden enkele belangrijke aspecten in verband met de potentiële rol van benthische protozoa als consumenten van de aanwezige benthische koolstofbronnen (hoofdstukken 4 & 5) en als voedselbron voor hogere trofische niveaus (hoofdstuk 6) bestudeerd door middel van experimenten.

stroomsnelheden die mogelijke uitspoeling van de ciliaten veroorzaken in het zandige station. Dit zandige station was namelijk helemaal tegen de laagwaterlijn gelegen. Gezien de samenstelling van de ciliatengemeenschap in Appels en hun verticaal distributiepatroon is het onduidelijk of de ciliaten in Appels echte benthische ciliaten zijn. Het is mogelijk dat de meeste ciliaten in Appels geassocieerd zijn met detritus partikels die er bezinken en terug in suspensie komen gedurende een getijdencyclus. Op de Molenplaat werd een totaal andere en veel rijkere ciliatengemeenschap gevonden met 107 niet-vastgehechte taxa die behoorden tot ten minste 52 genera en 15 ordes. De ciliaten densiteiten waren ook hoger op deze locatie en in tegenstelling tot Appels vertoonden ze een duidelijk seizoenaal patroon. In het slibrijke station nam de ciliaten densiteit sterk af na de winter toen de accumulatie van slib er begon. De rijke ciliatengemeenschap die hier aan het sedimentoppervlak te vinden was in de winter verdween haast naar de zomer toe, terwijl de ciliatengemeenschap onder het sedimentoppervlak arm was en weinig veranderde in de loop van het jaar. De soortenrijkdom en de densiteit van de ciliatengemeenschap waren hoger in het zandige station. Bovendien waren seizoenale en verticale patronen hier minder uitgesproken. Ciliaten densiteiten in het zandige station veranderden geleidelijk van een minimum in de winter naar een zomer maximum. Tegelijkertijd verschoof het verticale verspreidingspatroon naar boven. De ruimtelijke en temporele verspreidingspatronen van de ciliaten op de Molenplaat suggereren dat sedimentkarakteristieken een belangrijke factor zijn voor de ciliaten op deze locatie. De waargenomen verschillen tussen het zandige en slibrijke station op de Molenplaat en de seizoenale patronen op het slibrijke station tonen aan dat fysieke eigenschappen van het sediment belangrijker waren voor de ciliaten dan de beschikbaarheid van voedsel of de temperatuur. De resultaten van het zandige station suggereren dat de temperatuur en de beschikbaarheid van voedsel en zuurstof waarschijnlijk betrokken zijn in de regulatie van seizoenale en verticale distributiepatronen van de ciliaten wanneer de fysieke eigenschappen van het sediment niet beperkend zijn.

De rol van protozoa

Het belang van protozoa in vergelijking met andere grazers

Het relatief belang van protozoa en meio- en macrobenthische consumenten in de koolstof- en energiestroom in sedimenten is amper bestudeerd. De biomassa van de protozoa geeft een eerste indruk over hun belang. De biomassa van de protozoa bekomen tijdens deze studie werd vergeleken met gegevens over de biomassa van metazoa tijdens het einde van de lente (Molenplaat; P. M. J. Herman, M. Steyaert pers. med.) of het begin van de herfst (Appels; J. Seys pers. med.). In het zandige station te Appels was de biomassa van de protozoa groter dan die van de metazoa, terwijl de biomassa van de protozoa in de andere stations klein was (< 5 %) in vergelijking met de biomassa van meio- en macrobenthos. Aangezien kleine cellen hogere groeisnelheden hebben is het relatief metabolisch belang van protozoa echter hoger dan verwacht op basis van hun biomassa. De biomassa van protozoa en metazoa in de 4 stations aan het einde van de lente/begin van de herfst en schattingen van hun gewichtsspecifieke metabolische snelheden suggereren dat protozoa instaan voor 29 tot 96 % van de totale metabolische snelheid van de benthische consumenten. De geschatte relatieve metabolische snelheid van de protozoa was hoger in de zandige dan in de slibrijke stations in Appels zowel als op

in het slibrijke station. Er zijn bovendien weinig aanwijzingen dat meio- of macrofauna de bacteriële densiteiten in sedimenten kunnen reduceren of controleren (Kemp 1990). Samenvattend lijkt het erop dat in slibrijke sedimenten die een hoge bacteriële productie hebben het grootste deel van deze productie onaangeroerd blijft door grazers. Het is mogelijk dat een belangrijk deel van de bacteriële productie in bepaalde zandige sedimenten wel begraasd wordt.

Protozoa kunnen niet alleen een kwantitatieve invloed uitoefenen op de gemeenschappen van hun prooien maar kunnen ook de samenstelling van deze gemeenschappen beïnvloeden aangezien vele protozoa selectieve predatoren zijn (Verity 1991). De impact van die selectieve begrazing hangt grotendeels af van het selectiemechanisme en van het relatief belang van actieve en passieve selectie. In deze studie werd het gedrag van een aantal ciliatensoorten geobserveerd om na te gaan op welke manier ze diatomeeën selecteren in mengsels van diatomeeënsoorten. Benthische diatomeeën zijn belangrijke primaire producenten en belangrijke voedselbronnen in estuaria, maar de impact van algivorie door benthische protozoa is amper onderzocht. Voor de experimenten werden 3 ciliatensoorten gebruikt, namelijk 3 *Strombidium* soorten en een *Pseudochilodonopsis* soort, en 3 diatomeeënsoorten van intertidale sedimenten van het Westerschelde estuarium. In elk experiment werd een mengsel van 2 diatomeeënsoorten aan 1 ciliatensoort aangeboden. Er werd bepaald welke diatomeeënsoort bij voorkeur werd gegeten. Bovendien werd het voedingsproces onderverdeeld in 3 stappen, namelijk het stoppen van de ciliaat bij een diatomee ('encounter'), de poging om deze diatomee op te nemen ('attack') en de uiteindelijke opname ('capture'). De verschillen tussen de 2 prooisoorten voor deze 3 stappen werden bestudeerd. De resultaten van deze studie suggereren dat trofische interacties tussen ciliaten en diatomeeën veel complexer zijn dan over het algemeen wordt aangenomen. De experimenten tonen ontegensprekelijk aan dat de ciliaten zeer selectief zijn en een onderscheidt maken tussen gelijkaardige en fylogenetisch sterk verwante diatomeeënsoorten in mengsels. De prooivoorkeur was ook sterk predator-specifiek en werd niet beïnvloed door de densiteit van de prooien, noch door de prooisoort waarop de ciliaten voorheen werden opgekweekt. Bovendien veranderde de voorkeur van de ciliaten niet als de relatieve densiteit van de prooisoorten veranderde. De observaties suggereren dat passieve selectie, die bepaald wordt door de relatieve beschikbaarheid en de relatieve vatbaarheid voor predatie van de diatomeeënsoorten, slechts een ondergeschikte rol speelde in de predatie van diatomeeën door de ciliaten in deze studie. De prooivoorkeur van de ciliaten bleek vooral het resultaat te zijn van actieve selectie tijdens de 'encounter' stap, en in mindere mate ook tijdens de 'attack' stap van het voedingsproces. Bovendien suggereren de resultaten de selectie van de 'encounters' gebeurde voordat er contact was met de diatomeeën, tenminste bij de *Strombidium* soorten. Bijkomende experimenten met 'T-mazen' bevestigen dat deze ciliaten in staat zijn een onderscheid te maken tussen diatomeeënsoorten op basis van opgeloste chemische verbindingen. Samen met de resultaten van de observaties suggereren deze experimenten dat soortspecifieke opgeloste chemische verbindingen betrokken waren bij de selectie van individuele diatomeeën uit de mengsels, tenminste voor de *Strombidium* soorten. Doordat de trofische interacties tussen ciliaten en diatomeeën zeer gespecialiseerd zijn, zoals deze experimenten aantonen, kan algivorie door ciliaten in belangrijke mate de structuur en de diversiteit van benthische diatomeeëngemeenschappen beïnvloeden. Bovendien moet er met de uitgesproken specificiteit van algivorie door ciliaten en de herkenning van chemische verbindingen die door diatomeeën uitgescheiden worden rekening gehouden worden bij het opzetten van begrazingsexperimenten.

suggereren dat predatorische nematoden een aanzienlijk deel van de ciliaten productie in fijnzandige sedimenten van de Molenplaat kunnen consumeren. Anderzijds is er geen kwantitatieve informatie beschikbaar over de trofische interactie tussen benthische 'deposit-feeders' en protozoa. Dit onderwerp dient verder onderzocht te worden want 'deposit-feeders' zijn een belangrijke groep van metazoa in intertidale estuariene sedimenten. Een belangrijk deel van de nematoden (vooral in het slibrijk station) en van de macrofauna op de Molenplaat (Herman et al. 2000, M. Steyaert pers. med.) is 'depost-feeder', terwijl nagenoeg alle metazoa in Appels 'deposit-feeders' zijn (oligochaeten; Seys et al. 1999). Een ander aspect dat niet aan bod kwam in deze studie is de voedingswaarde van protozoa voor meio- en macrobenthos. Dit onderwerp is amper bestudeerd en is wellicht vooral belangrijk om het belang van protozoa voor 'deposit-feeders' te evalueren. Aangezien 'deposit-feeders' vrij onselectief grazen en de biomassa van andere voedselbronnen zoals detritus, bacteriën en algen hoger is dan die van de protozoa, is het voorkomen van essentiële nutriënten in protozoa (bv. poly-onverzadigde vetzuren; Zhukova & Kharlamenko 1999) een belangrijk gegeven dat verder onderzoek verdient.

Deze studie benadrukt het belang van kleine ($\leq$ 20 μm) protozoa (de nano-heterotrofen, zoals ze hier genoemd werden) in sedimenten. Doordat ze hoge densiteiten en hoge groeisnelheden hebben kunnen ze evenveel of zelf meer bijdragen tot het benthische metabolisme dan metazoa, tenminste in de sedimenten die in deze studie bestudeerd werden. Deze kleine protozoa kunnen een aanzienlijk aandeel van de bacteriële productie begrazen in fijnzandige sedimenten met een lage concentratie aan organisch materiaal (zoals het zandige station op de Molenplaat). Toch ondersteunen de resultaten van deze studie het idee dat protozoa waarschijnlijk geen belangrijke consumenten zijn van de bacteriële productie in de meeste aquatische sedimenten. Aangezien noch protozoa noch metazoa de bacteriële gemeenschappen lijken te controleren moeten alternatieve oorzaken van bacteriële mortaliteit zoals de lyse van bacteriële door virussen, die tot nu toe vooral in pelagische ecosystemen bestudeerd werd (e.g., Noble & Fuhrman 2000, Pedrós-Alió et al. 2000), onderzocht worden. Anderzijds houden de klassieke technieken die momenteel gebruikt worden voor het kwantificeren van bacterivorie door protozoa geen rekening met de mogelijke invloed van selectieve begrazing. De selectieve begrazing van metabolisch actieve bacteriën door protozoa (González et al. 1993, del Giorgio et al. 1996) is bijvoorbeeld een onderwerp dat nader onderzocht dient te worden. De experimenten in verband met selectieve begrazing van diatomeeën door ciliaten in deze studie tonen aan de trofische interactie tussen diatomeeën en ciliaten alvast zeer complex is.

Deze studie levert informatie over een beperkt aantal aspecten in verband met de rol van protozoa in intertidale estuariene sedimenten. Vele andere interessante aspecten konden niet onderzocht worden door tijdsbeperkingen, maar soms ook omdat er geen geschikte methoden voorhanden zijn. Verdere inspanningen zijn nodig om deze methodologische problemen op te lossen.

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Definitions

amoebae: unicellular non-photosynthetic wall-less protists whose shape is subject to constant change due to formation and retraction of pseudopodia (Lawrence 1995).

benthos: those organisms attached to, living on, in or near the sea bed, river bed or lake floor (Lincoln et al. 1998).

brackish: pertaining to water of salinity intermediate between fresh water and sea water (Lincoln et al. 1998).

ciliates: free-living and sessile protozoans of complex cellular structure, bearing cilia, often in rows on the surface or grouped into compound structures (Lawrence 1995).

DAPI: 4',6-Diamidino-2-Phenylindole, a blue-fluorescent UV excited dye that binds to DNA.

deposit feeder: any organism feeding on fragmented particulate organic matter in or on the substratum (Lincoln et al. 1998).

estuary: an inlet of the sea, within which seawater is measurably diluted with freshwater derived from land drainage, and which reaches into a river valley as far as the upper limit of tidal rise (after McLusky 1993). **Coastal plain estuaries** are by far the commonest type of estuaries and conform most closely to the classical concept of an estuary (Boaden & Seed 1985). They were formed at the end of the last ice age as the rising seawater level invaded low-lying coastal river valleys.

- **classification of estuarine divisions according to salinity** (McLusky 1993):
  tidal freshwater reaches: salinity < 0.5
  oligohaline reaches: salinity 0.5 to 5
  mesohaline reaches: salinity 5 to 18
  polyhaline reaches: salinity 18 to 30
  euhaline reaches: salinity > 30
- **classification of estuaries according to the tidal range** (Little 2000):
  microtidal: with a tidal range < 2 m
  mesotidal: with a tidal range of 2 to 4 m
  macrotidal: with a tidal range > 4 m

filter feeder: any animal that feeds by filtering suspended particulate organic matter from water (Lincoln et al. 1998).

flagellates: highly diverse group of unicellular eukaryotic microorganisms, including photosynthetic and non-photosynthetic, heterotrophic species. They are motile in the adult stage, swimming by means of flagella (Lawrence 1995).

heterotrophic: obtaining nourishment from exogenous organic material; used for organisms unable to synthesize organic compound from inorganic substances (Lincoln et al. 1998).

interstitial: pertaining to, or occurring within, the pore spaces (interstices) between sediment particles (Lincoln et al. 1998).

intertidal: between high- and low-water marks (Lawrence 1995).

isopycnic: having the same density (Lincoln et al. 1998).

microbenthos: microscopic benthic organisms less than 0.1 mm in length (Lincoln et al. 1998).

meiobenthos: small benthic organisms that pass through a 1 mm mesh sieve but are retained by a 0.1 mm mesh (Lincoln et al. 1998).

macrobenthos: the larger organisms of the benthos, exceeding 1 mm in length (Lincoln et al. 1998).

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